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ON THE FAMILY OF ACHIRIDAE OR BROAD-SOLES, WITH DESCRIPTION OF A NEW SPECIES ACHIRUS BARNHARTI FROM CALIFORNIA

BY

DAVID STARR JORDAN

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ON THE FAMILY OF ACHIRIDAE OR BROAD-SOLES, WITH DESCRIPTION OF A NEW SPECIES ACHIRUS BARNHARTI FROM CALIFORNIA

BY

DAVID STARR JORDAN

The order of Heterosomata, or flat-fishes, is unique among vertebrate animals in having the anterior part of the cranium so twisted that both eyes are on the same side of the body. In all the species, the body is much compressed, in the adult state lying flat on the sea bottom, both eyes being on the side that remains uppermost. In the larval condition the eyes are symmetrical, the body translucent and swimming upright in the water. But with development, the eye of the one side is transferred to the other and the unsymmetrical condition appears. The species inhabit sandy bottoms in nearly all seas, a few ascending rivers. The position of the eyes and color is constant in most, but not all groups. In general, northern forms have the eyes and color on the right side and show an increased number of vertebrae. In the tropical forms, the eyes and color are mostly on the left.

The order contains two widely distinct groups, the flounders and the soles, each of these in recent classification composed of a few distinct families.

The soles are much more specialized than the flounders and are mainly confined to the warm seas. In all, the eyes are small, close together, in the same orbit or separated by a bony ridge; the teeth are minute or obsolete; the very small mouth is twisted toward the blind side; the edge of the preopercle is adnate, concealed by skin and scales; the gill membranes are joined to the shoulder girdle above or to the isthmus below, restricting the apertures. The fin rays of dorsal and anal are very numerous, all jointed; the pectoral fins are small or wanting; the ventral fins small, thoracic in position or sometimes wanting.

The earliest known soles are from the European Miocene. The group has diverged widely from that of the flounders which are its probable ancestors. The problem of the origin of the Heterosomata is still unsettled, but the thoracic position of the ventral fins and the number of their rays may indicate an approximation to the ancestors of the spiny rayed fishes.

The earliest known flounders belonged to the Eocene (*Bothus minimus*) and Miocene (*Zororhombus: Evesthes*) the two first referred to the Bothidae, the last to the Paralichthyidae. But it is evident that the origin of the group goes much farther back.

The present writer (1922, p. 169) divides the soles into four families. The Achiridae or Broad-soles have the eyes and color on the right side separated by a bony ridge and the ventral fin on the right side with a wide base continued along the ridge of the abdomen; pectoral fin small or wanting; caudal fin free from dorsal and anal (rarely slightly connected); vertebrae relatively few, about 28. The species are with one exception American and center in the tropics.

The Soleidae or true soles have the eyes and color on the right side, the eyes separated by a bony ridge and the caudal free from dorsal and anal, but the ventral fins are not extended at base and not connected with the anal fin. Pectoral of right side moderately developed, that of blind side often wanting. Vertebrae numerous. 44 to 50. The species are nearly all European, especially characteristic of the Mediterranean fauna.

The Synapturidae or Zebra-soles differ from the Soleidae in having the dorsal and anal fully united with the caudal, meeting around the tail. The body is broadly elliptical in form. Vertebrae numerous, about 50. The species are mostly Asiatic.

The Cynoglossidae or Tongue-soles have the eyes and color on the left side; both eyes in the same socket, without bony ridge; the dorsal and anal joined to the caudal; pectorals wanting; ventrals, if present, free from the anal; body elongate; vertebrae numerous, about 50. The species are largely Asiatic, but some occur on each coast of America and in southern Europe.

Family ACHIRIDAE

The Broad-Soles

Soles with eyes and color on right side and separated by distinct bony ridge; the ventral with broad base confluent with the anal. Body oblong or ovate, with the color on the right side; eyes moderate or small, upper eye usually more or less in advance of lower; mouth small, more or less twisted toward the blind side; teeth little developed, in villiform bands if present; edge of preopercle adnate, usually concealed by the scales; gill openings more or less narrowed, gill membranes adnate to shoulder girdle above, blind side of head usually with fringes; pectoral fins small, sometimes wanting; ventral fins developed, one or both of them sometimes obsolete; scales usually ctenoid, rarely wanting; lateral line single, straight.

This group contains three widely divergent subfamilies, one of them, the Apionichthyinae, perhaps deserving of recognition as a distinct family, its character of restricted gill openings showing an even wider divergence from the usual type than do the Achirinae from the true soles. The soles of this group, very rare in collections, appear to be fresh-water fishes of the streams of Brazil, and degenerate offshoots of Achirus. The Asiatic genus *Amate* resembles some of the Soleinae (*Aseraggodes* and others) very closely, differing in the technical character of the extended base of the ventrals.

Pending fuller investigation, we regard the Apionichthyinae and Gymnachirinae as subfamilies of Achiridae.

The American species of this family are described by Jordan and Goss in "A Review of the Flounders and Soles (Pleuronectidae) of America and Europe; Rept. Comm. Fish. and Fisheries for 1886" (1889). The detailed synonymy of each species is given in this paper.

ANALYSIS OF GENERA OF ACHIRIDAE

a. Gill opening not closed below, not reduced to a lateral slit behind the opercle; eyes not very small, as wide as the interspace between them.

b. GYMNACHIRINAE. Body and fins entirely scaleless; pectoral of two rays only or wanting; jaws and vertical fins enveloped in thick skin; left side of head with a network of fringes; body deep, the depth half the length; teeth wanting ....*Gymnachirus*

bb. ACHIRINAE. Body covered with scales, ctenoid, at least on the eyed side; vertical fins scaly; jaws not enveloped in thick skin; both ventral fins present; vent to the left of the median line of the abdomen.

c. Body deep, the depth more than half the length to base of caudal; scales ctenoid, those on head more or less enlarged, those on its blind side with fringes; teeth present, very small; pectoral fins small or wanting .... *Achirus*

cc. Body oblong-ovate, the depth less than half the length to base of caudal; snout prolonged, hooked over the lower jaw; scales of head little enlarged; chin fringed; pectoral fins wanting; scales cycloid .... *Amate*

aa. APIONICHTHYINAE. Gill openings reduced to a short lateral slit on side of opercle; body oblong ovate, covered with small ctenoid scales; snout produced, curving around the lower jaw; blind side of head with the scales mostly reduced to fringes; teeth minute; eyes small.

d. Eyes not minute, about as wide as interspace; dorsal and anal entirely free from caudal.

e. Left ventral fin developed; body oblong, the depth about two-fifths the length.

f. Gill openings present on both sides .... *Achiropsis*

ff. Gill openings present on blind side only .... *Pnictes*

ee. Left ventral wanting; gill openings present on both sides.. *Soleonasus*

dd. Eyes reduced to minute points; scales very small; dorsal and anal more or less connected with the pointed caudal; body elongate, the depth more than two-fifths length; left ventral minute or wanting .... *Apionichthys*

Subfamily GYMNACHIRINAE

Gymnachirus Kaup

Gymnachirus Kaup, Uebers. Soleinae, Wiegmann's Archiv, 1858, 101.
Haplotype.—Gymnachirus nudus Kaup.

Of this singular genus, two species have been recorded, *Gymnachirus nudus* Kaup from Brazil, and *Gymnachirus fasciatus* Günther. from unknown locality, probably South America. *G. nudus* is said to have the dorsal rays 51, the anal 42, and the pectoral fins wanting (or overlooked?); *G. fasciatus* has dorsal rays 68, the anal 50, and a very small pectoral fin on the right side, of two rays only, one-third length of eye. In both, the body has fourteen black cross-bars, the caudal with two or three black bars.

Subfamily ACHIRINAE

Achirus Lacépède

Achirus Lacépède, Hist. Nat. Poiss., 4, 659, 1803. (*Achirus fasciatus*, etc.)
Trinectes Rafinesque, Atlantic Journal, etc., 1, 1832 (*scabra* = *fasciatus*).
Grammichthys Kaup. Wiegmann's Archiv, 1858, 94.
(*lineatus*, etc., *Achirus* being restricted to *Paradachirus barbatus*, one of the Soleinae.)
Monochirus Kaup, *loc. cit.* (*maculipinnis* = *lineatus*) (not of Rafinesque, Cuvier, or Bonaparte).
Baiostoma Bean, Proc. U. S. Nat. Mus., 1882, 413 (*brachiale* = *lineatus*).
Type (by tautonomy).—*Pleuronectes achirus* L. = *Solea gronovii* Günther.

The species with small pectoral fins on one or both sides may be separated subgenerically from those in which there is no trace of these organs. The numerous species representing the three groups are however closely related and all belong to the American fauna, being most numerous in the tropics. Some of them at least ascend the rivers.

As the original *Pleuronectes achirus*, which by tautonymy must be taken as the type of the genus *Achirus*, seems to have been a species with small pectorals on both sides, we adopt the name *Achirus* for the subgenus with two pectorals. Jordan and Goss (1889) have indicated *Achirus fasciatus* as the type of *Achirus*, as Lacépède wrongly thought *Pleuronectes achirus* synonymous with his *fasciatus*. Most of the species referred to *Achirus* by Jordan and Goss belong to the typical section or subgenus *Achirus*.

a. ACHIRUS. Pectoral fin present on both sides, that of the left side rudimentary, of a single ray, that of the right side with about 3; scales small; fin rays relatively numerous.

b. Dorsal rays 60 to 68; anal 48; scales 80, depth 1⅖ in length; blind side of head little fringed; body and fins irregularly spotted with dark and with ten narrow dark cross streaks .... *achirus*

bb. Dorsal rays 53 to 57; anal rays 40 to 42; depth 1⅗ in length; blind side of head much fringed; sides with hair-like filaments; head, body, dorsal and anal with a network of dark lines with traces of cross-streaks; caudal nearly plain .... *inscriptus*

aa. GRAMMICHTHYS. Pectoral of right side only present.* of one to 6 rays.

* The small pectoral fin is occasionally obsolete in the adult of *A. fonsecensis*.

c. Dorsal fin long, of 65 or 66 rays; anal rays 44.

d. Pectoral rays six; no hair-like filaments; dorsal rays 62 to 65; anal rays 46 to 51; scales 71 to 76; depth $1\frac{5}{7}$ in length; body with 10 to 12 very narrow dark cross-lines; these often obscure; fins vaguely spotted .... *klunzingeri*

dd. Pectoral rays about 2; dorsal rays 66; anal 49; scales 70; depth 1⅖ in length; chin prominent, protruding beyond upper jaw; scales with many black hair-like filaments; pectoral black; sides with many obscure dark clouds and traces of cross-streaks; no small spots .... *mentalis*

cc. Dorsal fin shorter, of 50 to 61 rays, anal 35 to 47.

e. Pectoral fin longer than eye, of 4 to 6 rays, body with 8 to 10 narrow vertical cross-bars, faint with age; depth 1½ in length.

f. Vertical fins all with round black spots, usually most distinct on caudal; all growing faint or obsolete with age; scales with hair-like appendages;

g. Scales 75 to 85; dorsal rays 49 to 57; anal 37 to 44 .... *lineatus*

gg. Scales 55 to 67; dorsal rays 50; anal 30 .... *comifer*

ff. Vertical fins dark without distinct markings; body with 8 or 9 faint cross-streaks; hair-like appendages relatively few; dorsal rays 52 to 56; anal 40 to 43; scales 54 to 61 .... *mazatlanus* ee. Pectoral fin of 2 or 3 short rays only, not longer than eye.

h. Body with 6 to 12 dark cross-bands, obsolete with age.

i. Body narrowly ovate, the depth 1⅚ in length; dorsal rays 57 to 61; anal 42 to 45; scales 60 to 65 .... *fonsecensis*

ii. Body broadly ovate, the depth 1⅔ in length; hair-like appendages few; dorsal rays 56; anal 39; scales 76; fins faintly streaked, scales finely punctulate .... *punctifer*

iii. Body broad, depth 1¾ in length; pectoral very small, sometimes wanting; preopercle scaleless; hairs few; cross-bars 8 to 10; spots few; dorsal rays 56; anal 43; scales 72 .... *paulistanus*

hh. Body with numerous (20 to 40) dark cross-bars, at least as wide as the pale interspaces.

j. Blind side of head scantily fringed; head and body with 13 to 20 narrow gray wavy bars, narrower than the interspaces; on a dark background; caudal with black spots; dorsal rays 53 to 56; anal 42 to 44; scales 70 to 80; depth 1½ in length .... *scutum*

jj. Blind side of head profusely fringed; hair-like appendages numerous; dorsal rays fringed; vertical fins with three oblong black spots on the interspaces between rays; dorsal rays 55; anal 47; scales 77 to 80; depth 1⅔ in length.

aaa. TRINECTES. Pectoral fins wholly wanting, at all stages of development. (A rudimentary fin of a single ray two-thirds as long as the eye was noticed in one example from Pensacola. See Jordan and Goss, 1889, p. 316.)

k. Dorsal rays 46; anal 33; scales 70; right lower lip with serrated fringes; nostril in a fringed tube; depth 1½ in length; head and body with round or kidney-shaped pale spots, edged with dark brown .... *fimbriatus*

kk. Dorsal rays 50 to 55; anal 37 to 46; scales 66 to 75; no pale spots.

l. Body broad-ovate, the depth 1⅘ in length to base of caudal; hair-like appendages wanting or sparse; fringes on head relatively few; body with about eight dark cross-bars, which vary much in width; vertical fins with dark blotches on the membranes; caudal with oblong dark spots .... *fasciatus*

m. Blind side with round dark spots .... Subspecies *fasciatus*

mm. Blind side immaculate; dark bars broader and more numerous .... Subspecies *browni*

ll. Body almost circular, the depth 1⅓ in length; numerous fringes on lips about nostril and gill opening; tips of anterior dorsal fringed; sides with numerous hair-like appendages. Color brown, uniform in the adult; blind side immaculate .... *barnharti*

kkk. Dorsal rays 60; anal rays 41; depth 1½ in length; nasal barbel fringed; fringes on head few; sides with hair-like appendages. Color pale with scattered black points and blotches.
m. Scales 80; cross-streaks obscure .... *jenynsi*
mm. Scales 57; six black cross-bars .... *errans*

Subgenus ACHIRUS

Achirus achirus (Linnaeus)

Pleuronectes achirus Linnaeus, Syst. Nat., ed. X, 268, 1758.
(Surinam, after Gronow, *Pleuronectes oculis dextris, pinnis pectoralibus nullis;* dorsal rays 60; anal 48.)
Solea gronovii Günther, Cat., 4, 472, 1862.
(Surinam; Demarara, West Indies.)

I find it necessary to identify *Pleuronectes achirus* with Gronow's fish, as no other found in Surinam has so large a number of fin rays. The very small pectorals were, no doubt, overlooked by Gronow. The color agrees with that given in Gronow's account. We have not seen this species, and it may be founded on a faded example of *A. inscriptus*. The name *achirus* has priority.

Achirus inscriptus Gosse

Achirus inscriptus Gosse, Nat. Sojourn Jamaica, 51, pl. 1, f. 4, 1852 (Jamaica).
Monochir reticulatus Poey, Memorias, 2, 317, 1862 (Havana).

West Indies, north to Key West. Specimens seen from Key West and Hayti.

Subgenus GRAMMICHTHYS

Achirus klunzingeri (Steindachner)

Solea klungzingeri Steindachner, Fische der Cauca., etc., 44, 1870 (Guayaquil).
Achirus klunzingeri Gilbert and Starks, Fishes of Panama, Mem. Calif. Acad. Sci., 4, 200, 1904 (Panama).

Known from Ecuador and Panama. Gilbert and Starks found it abundant in Panama Bay.

Achirus mentalis Günther

Solea mentalis Günther, Cat. 4, 475, 1862 (Para).

Coast of Brazil: I have examined a specimen from Pará.

Achirus lineatus Linnaeus

Pleuronectes lineatus Linneaus, Syst. Nat., ed. X, 268, 1758; based on Browne and Sloane, both records from Jamaica, not of edition XII, which is *Achirus fasciatus*, from Charleston.

Monochir maculipinnis Agassiz, Pisc. Brasil., 88, pl. 49, 1829 (Brazil).

?*Achirus lorentzi* Weyenbergh, Act. Acad. Sci., Buenos Aires, **3**, 13, 1877 (Parana R.; streams of Uruguay). Jordan, Proc. Acad. Nat. Sci. Phila., **1887**, 389 (copy of Weyenbergh's description).

Baiostoma brachialis Bean, Proc. U. S. Nat. Mus., **1882**, 413 (Pensacola).

This is the most abundant species of sole from southern Florida southward. It is subject to great variation in color as well as in numbers of fin rays and scales. We have examined many specimens from Egmont Key, Pensacola, and Cienfuegos, besides twenty-nine ports of Brazil, these last in the Museum of Comparative Zoology at Cambridge, most of them collected by Agassiz.

The description of *Achirus lorentzi* shows no tangible difference from *Achirus lineatus* unless it be in the number of dorsal rays, which are given as 40, the anal as 38; the first figure is apparently a misprint.

Achirus comifer Jordan and Gilbert

Achirus comifer Jordan and Gilbert, Proc. U. S. Nat. Mus., 1884, 31 (Key West).

The type of this nominal species from Key West shows fin rays and scales in smaller number than in any example of *Achirus lineatus*. It probably represents a local variation rather than a distinct species.

Achirus mazatlanus (Steindachner)

Solea mazatlana Steindachner, Ichth. Notiz., **9**, 23, 1869 (Mazatlan).

Solea pilosa Peters, Berlin, Monatsber, **1869**, 709 (Mazatlan). West coast of Mexico, common about Mazatlan and recorded from Chiapas.

Achirus fonsecensis (Günther)

Solea fonsecensis Günther, Cat. **4**, 475, 1862 (Gulf of Fonseca).

Achirus fonsecensis Gilbert and Starks, Fishes of Panama, Mem. Calif. Acad. Sci., **4**, 201, 1904 (Panama).

Solea panamensis Steindachner, Ichth. Beitr., **5**, 10, 1877 (Panama).

Solea fischeri Steindachner, Flussfische Sud Amer., 13, 1879 (Rio Mamone, Panama). (According to Gilbert and Starks, *Achirus fischeri* with one pectoral ray is the young of *Achirus fonsecensis* and *Achirus panamensis* without pectorals is the adult.)

West coast of Mexico and Central America, Mazatlan to Panama. Abundant at Panama.

Achirus punctifer (Castelnau)

Monochir punctifer Castelnau, Anim. Amer. du Sud, 80, pl. 41, fig. 3, 1855 (Rio de Janeiro).

Coast of Brazil.

A specimen from Itabapuana (11436 M. C. Z.) is referred by Jordan and Goss (1889) to this species, although the pepper-like spots are much exaggerated in Castelnau's figure.

Achirus paulistanus Ribeiro

Achirus paulistanus Miranda-Ribeiro, Archivios, **18**, 25, 1915 (Saõ Paolo).

Achirus scutum (Günther)

Solea scutum Günther, Cat. **4**, 475, 1862 (Gulf of Fonseca).
Achirus scutum Gilbert and Starks, Fishes of Panama, Mem. Calif. Acad. Sci., **4**, 202, 1904 (Panama).

West coast of Central America, abundant in Panama Bay.

Achirus garmani Jordan

Achirus garmani Jordan, Rev. Flounders and Soles, 314, 1889 (Rio Grande do Sul).

Coast of Brazil: known only from the type (11246 M. C. Z.).

Subgenus TRINECTES

Achirus fimbriatus (Günther).
Solea fimbriata Günther, Cat. **4**, 477, 1862 (Gulf of Fonseca).

West coast of Nicaragua. Known as yet solely from the original type.

Achirus fasciatus Lacépède

American Sole, Hog-choker

Pleuronectes lineatus Linnaeus, Syst. Nat., ed. XII, 458, 1766 (on a specimen from Charleston, coll. Dr. Garden—not *Pleuronectes lineatus* ed. X, which is based on Gronow).
Achirus lineatus Cuvier, Règne Animal, and of many authors.
Achirus fasciatus Lacépède, Hist. Nat. Poiss., **4**, 659, 662, 1803 (exclusive of synonymy, the description taken from Linnaeus' account of the Garden specimen).
Achirus mollis Storer, Synopsis, 228, 1846 (Charles River; Holmes Hole).
Pleuronectes apoda Mitchill, Amer. Monthly Mag., Feb'y, 1818; 244 (Straits of Bahama).
Trinectes scabra Rafinesque, Atlantic Journal, **1**, 1832 (Pennsylvania, in fresh waters).
Solea browni Günther, Cat. **4**, 477, 1862 (New Orleans, Texas).

Massachusetts to Texas, generally common, often ascending streams, the only species found north of the Florida Keys. Northward this sole has the blind side marked with round dark spots of various sizes, while, on the Gulf coast, it has this side immaculate. At Charleston, the two types are equally abundant. One specimen from Boston has the left side unspotted. There seems to be no permanent character by which the spotless subspecies *browni* can be separated from *fasciatus*. This fish reaches a length of about six inches. Because of its small size it has little importance in the markets.

Achirus barnharti Jordan sp. nov.

Plate 1

Coast of southern California, Coronado Islands.

Head 3 ¾ in body; depth to base of caudal 1⅓; dorsal rays 55; anal 43; scales 68; pectorals none; ventrals of 5 rays, the right ventral continuous with the anal; dorsal and anal free from caudal; mouth moderate, reaching to front of lower eye; right lower lip fringed, upper less so; lower lip even with tip of snout, which is slightly bent downward over the mandible. Eyes small, the upper in advance; upper nostril in a wide smooth tube, nearer lower eye than tip of snout; lower nostril near tip of snout surrounded by fine papillae which are continuous with snout forming a papillated flap; strong dermal flaps on both sides at end of gill opening, one on lower side back of and above mouth; inter-orbital space almost twice diameter of eye and scaled over. Scales strongly ctenoid over entire body and fins, extending to tips of rays, much larger above eye; preopercle and below gill opening, anterior underside of head with numerous fringes; similar fringes extend to the tips of first 18 dorsal rays and lower side of ventral rays forming ridges; black hair-like appendages on right side, in groups, 4 above lateral line and 4 below, near to base of fins, some few scattered.

Color plain brown, vaguely clouded, fins dusky, narrowly edged with pale.

The type of this species, 8.06 inches long, was taken off the Coronado Islands in about 400 feet depth of water, by Percy S. Barnhart of the Scripps Institution for Biological Research, University of California, at La Jolla, California. It is in the Museum of Stanford University, No. 23240.

It is allied to *Achirus fasciatus*, the northernmost Atlantic species, differing from that and all others of the genus in the very deep body—almost circular in form. The hair-like appendages are much more developed than in *Achirus fasciatus*.

Achirus jenynsi (Günther)

Solea jenynsi Günther, Cat. 4, 476, 1862 (Rio de la Plata; after *Achirus lineatus* Jenyns).

Coast of temperate South America.

Of this species we have seen one example (11425 Mus. Comp. Zool.) from Rio Uruguay.

Achirus errans Ribeiro

Achirus errans Miranda-Ribeiro, Arch., 17, 23, 1915 (Rio de Janeiro; Paraguay).

This seems to differ from *A. jenynsi* in the larger scales.

Amate Jordan and Starks

Amate Jordan and Starks, Proc. U. S. Nat. Mus., 31, 228, 1906.
Type—Achirus japonicus Temminck and Schlegel.

In this genus, the pectoral fins are absent and the body oblong, much less broad than in *Achirus*, and the snout much more hooked above the mouth. The single known species, *Amate japonica* (Temminck and Schlegel) is rather common about the Inland Sea of Japan, where it is known as Sasa-ushino-shita.

Subfamily APIONICHTHYINAE

Achiropsis Steindachner

Achiropsis Steindachner, Ichth. Beitr., 5, 110, 1876.
Orthotype.—Solea nattereri Steindachner.

This interesting genus has a general resemblance to *Achirus*, but with the gill opening reduced to a small oblique slit near the upper margin of the opercle. The single species is recorded from Rio Negro in northern Brazil.

Dorsal rays 82; anal 61; v. 5–5; depth 2⅓ in length.

Pnictes Jordan

Pnictes Jordan, Proc. Acad. Nat. Sci. Phila., 1919, 343.
Orthotype.—Achiropsis asphyxiatus Jordan and Goss.

This genus agrees in most respects with *Achiropsis*, differing from that genus and all others in having no gill opening at all on the right or eyed side. The type specimen of *Pnictes asphyxiatus* (Jordan and Goss) (no. 11106, M. C. Z.) came from a stream at Goyaz in central Brazil, probably a branch of Rio Tocantins. Dorsal rays 69; anal 44; ventral 5–5; scales 70; depth 2⅓ in length.

Soleonasus Eigenmann

Soleonasus Eigenmann, Fresh Water Fishes of British Guiana, Mem. Carnegie Museum, **5**, 528, 1912.

Orthotype.—Soleonasus finis Eigenmann.

This genus is founded on two specimens of a little fish (type 2487, Carnegie Museum) from Tumutumari in British Guiana. The genus is close to *Apionichthys* but the left ventral is wanting, the body much deeper and the eye larger, as wide as the inter-orbital, and the caudal fin short. Dorsal rays in *S. finis* 64; anal 48; ventrals 5–0. Scales 80, depth 2½ in length. Sand color with faint cross streaks.

Apionichthys Kaup

Apionichthys Kaup, Wiegmann's Archiv, 104, 1858.

Soleotalpa Günther, Cat. **4**, 489, 1862 (unicolor).

Orthotype.—Apionichthys dumerili Kaup.

In this genus the left ventral is reduced to two or three rudimentary rays; the dorsal and anal are slightly connected with the caudal, which is lanceolate and a little longer than the head. The body in the typical species is comparatively elongate, the depth 2¾ in length; the eyes are very small, reduced to mere points, the scales very small, rough, those on head enlarged and fringed.

The type species *Apionichthys dumerili* Kaup (= *Soleotalpa unicolor* Günther, Cat. **4**, 489, 1862) is gray, the body and fins profusely covered with dark spots of varying sizes; dorsal rays 68 to 76; anal 56 or 57; ventrals 5–1; scales 86.

Soleotalpa unicolor from the "West Indies" is no doubt the same fish, although the color is described as "uniform brownish grey." The young example, 3 inches long, is no doubt faded. *Apionichthys nebulosus* Peters (1869, p. 709), from Surinam, is apparently also the same.

It seems to me that the name *dumerili* may be retained for this species, although no separate specific description was given and no locality assigned. The description of the species is included in Kaup's detailed account of the genus.

From Sicily, Steindachner (1868, p. 41) describes *Apionichthys ottonis*. It is clear brownish yellow: dorsal rays 70 to 73, anal 52 to 54, ventrals 5–0, scales 87 to 90, depth 2⅔ in length.

I have examined one specimen of *A. dumerili* from Obydos, Brazil (no. 4677, M. C. Z.).

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STANFORD UNIVERSITY, CALIFORNIA.

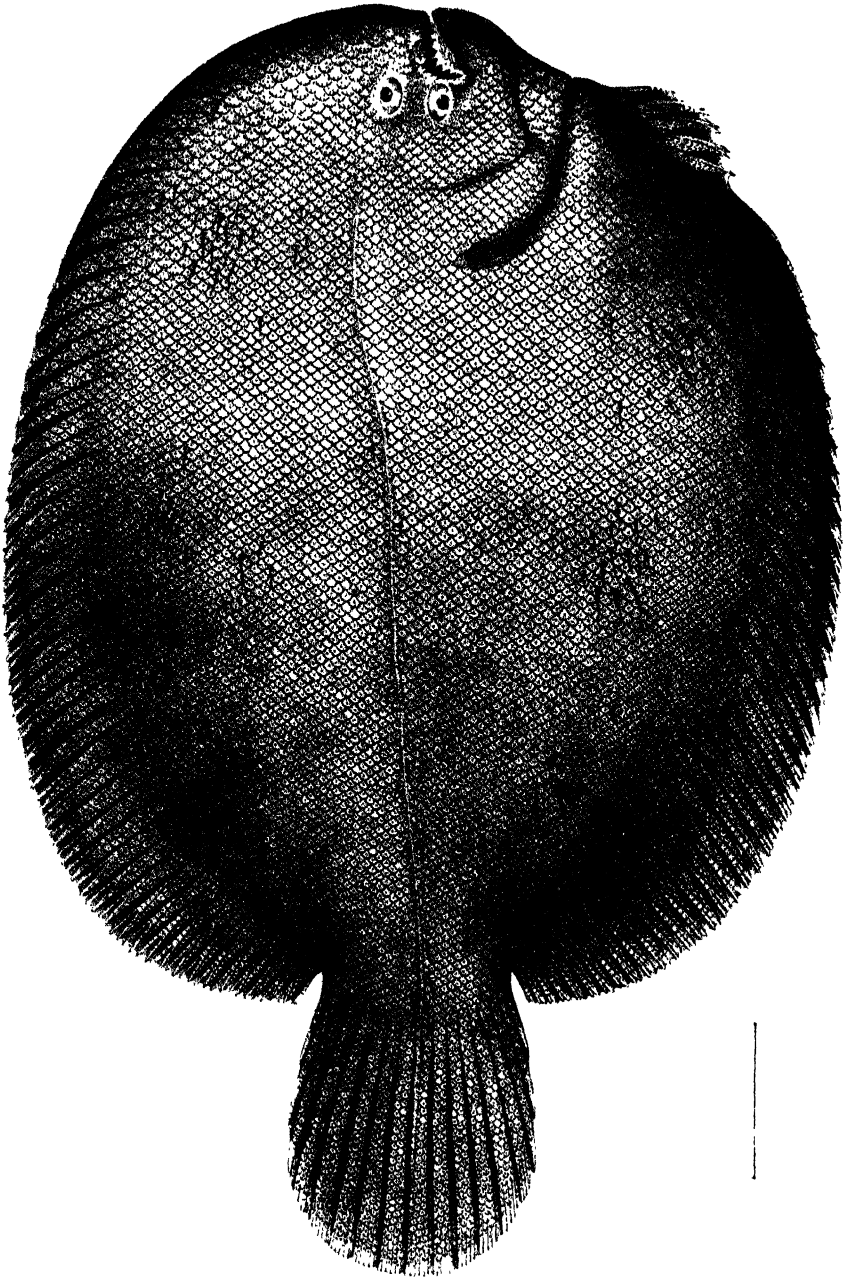
Transmitted June 4, 1923.

EXPLANATION OF PLATE 1

Achirus barnharti Jordan

Type: Coronado Islands.

Coll. Percy A. Barnhart. Drawn by William Sackston Atkinson. Scale, 1 inch.



A PRECIPITIN TEST IN EXPERIMENTAL AMOEBIC DYSENTERY IN CATS

BY

EDNA HANNIBAL WAGENER

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Issued January 26, 1924

A PRECIPITIN TEST IN EXPERIMENTAL AMOEBIC DYSENTERY IN CATS

BY

EDNA HANNIBAL WAGENER

The numerous chronic amoebic infections in man lead one to suspect the existence of a possible incomplete protective immunity on the part of the host against the invading organism. Izar (1914) obtained positive complement fixation reactions with serum from five human cases of amoebic dysentery, and with serum from three cats experimentally infected from man. He used in these tests aqueous antigens prepared from the faeces of infected cats or the pus from a liver abscess of man [?]. Normal faeces, on the other hand, or faeces from cases of bacillary dysentery used as controls, reacted negatively. Nor did the normal sera of man or cat react with either antigen. Hage (1920) attempted to repeat Izar's findings in human cases, using an antigen prepared from human faeces containing amoebae or the pus from a human liver abscess, but with negative results. The fact that he did not confirm Izar's findings can hardly be considered as conclusive, since the antigenic property is probably present only in minute quantities in either the faeces or in pus from a liver abscess of man. An attempt has been made by us to demonstrate a precipitin test in experimentally infected cats, using as an antigen scrapings of the amoebic ulcers of the colon of the cat.

Attempts were first made over a period of several months to infect young kittens rectally with motile forms of *Endamoeba dysenteriae* obtained from chronic cases of amoebiasis, but not a single infection occurred. On October 6 of this year, however, a stool specimen was obtained from an acute case of amoebic dysentery. The specimen was composed of bloody mucus in which were found numerous motile amoebae containing ingested red blood cells. Three newly weaned kittens were each given rectally, by means of a rubber catheter, 20 cc. of this specimen of faeces. One week after the infection, all three kittens developed a bloody dysentery. The bloody mucus from these kittens was frequently found to contain between 50 and 100 large, actively motile amoebae in a single microscopic field under the 1/6 objective. This material has since been used for transfers of the infection to other kittens. The strain has now had five passages with thirty infections in cats.

The cats used have varied from young kittens to adults. Unlike Baetjer and Sellards (1914), we have not found that the virulence of the strain of *Endamoeba dysenteriae* increased upon transfer from cat to cat, but rather that the age of the animal used and the amount of mucus with which it was infected influenced the length of the incubation period and the course of the infection. Young kittens were found to have an incubation period of two to five days and they frequently died on the fourth day of the infection, while adult cats have all had an incubation period of at least a week and live for fifteen days, or longer, after they develop definite symptoms of amoebic dysentery. The infection experiments were carried on jointly with Miss Margaret Thomson of the Zoological Laboratory under the direction of Professors Kofoid and Force.

Two adult cats developed chronic infections; they are still alive although amoebae have been observed in their stools for six weeks. One half-grown cat has apparently recovered and the stools have been free of amoebae for thirty days. All examinations for amoebae have, in every case, been made upon freshly evacuated faeces.

The animals have been closely watched during the course of the infection. When they appeared too weak to live through the night, they were given ether and bled from the hearts. The serum thus obtained was used for our serological tests. As the cats rarely eat during the last two days of their infection, the colon is free from faecal material and is found filled with mucus. The extensiveness of the ulceration depends largely upon the duration of the infection. Ordinarily, in kittens, only the rectum shows ulcers; but in an adult cat which had been infected for twenty-one days, the epithelium and even the mucosa were found eroded from the ilio-caecal valve to the anus.

In preparing the antigen, the ulcerated areas of the colon were removed aseptically, washed in three or four changes of sterile saline, and the ulcerated areas scraped with a sterile scalpel. These scrapings contain some epithelial cells, a few bacteria, and numerous amoebae. Smears of these scrapings in which the amoebae have been counted have been stained by Gram's method and a comparison of bacteria and amoebae made. Thus a slide which was found to average 150 amoebae to a field in ten fields counted averaged 200 bacteria in the same area and at the same magnification. A non-haemolytic, encapsulated *Streptococcus* is the most prevalent form of bacteria.

The scrapings from the ulcers were put immediately into Coca's extracting fluid, composed of 0.5 per cent sodium chloride, 0.05 per cent bicarbonate of soda, and 0.4 per cent phenol. This fluid is then covered with toluol. When shaken, the whole should be of a dilution which has the consistency of pea soup. Thus it is seen that, although the scrapings used for the antigen do not represent a pure culture of amoebae, the volume of protein derived from the amoebae is without question many times greater than that derived from the bacteria.

These scrapings were allowed to autolyze for several days, preferably a week, and the material was then filtered through hard filter paper. Sterile filter paper and glassware were used in order to prevent contamination. The scrapings from several cats were pooled before filtering to make the antigen as nearly as possible of standard strength.

A control to the antigen was prepared by culturing, both aerobically and anaerobically, the bacteria found in the scrapings. These cultures were grown for one week, after which they were killed with toluol and filtered through hard filter paper.

The blood obtained by bleeding the infected cats from the heart was permitted to clot and the serum drawn off. This serum was then used in the test, undiluted, and at dilutions of 1 to 1, 1 to 2, 1 to 4. and 1 to 8. The test was run in duplicate, the amoebic antigen being layered onto one series of dilutions of the serum, and the bacterial control onto the other series of dilutions of the serum. The tests were also controlled by using serum from a normal cat and by saline controls.

The tubes were then incubated at 37° C. in the water bath for one hour. They were then read, placed in the ice box, and read again the following morning.

Twenty-seven specimens of cat's blood have been tested with the following results. (See table on p. 18.)

Seven cats discharging amoebae for eight days or longer gave a strongly positive precipitin reaction up to 1 to 4 dilution of the serum with the amoebic antigen. Three cats sick from six to eight days gave a weakly positive reaction with undilute serum only.

Six cats dying *in less than six days* after they developed dysentery and five normal cats have given negative precipitin tests. The two cats which had chronic infections were bled from the ear, and when tested gave positive reactions only in the undilute serum. One cat dying with no lesions present gave a negative reaction. The recovered case is negative. In not one case did the bacterial control to the antigen give a positive precipitin test.

It is thus seen that the results obtained in the precipitin tests in amoebiasis are completely analogous to those obtained in bacterial diseases; namely, the cats sick for a week or longer gave positive precipitin tests while those sick for a shorter period of time gave negative tests. Normal cats gave no reaction.

To verify the fact that this reaction was not nonspecific, an extract was prepared from scrapings from the colon of a normal cat and this extract used as a control; the results were negative. The serum treated

TABLE SHOWING RESULTS OF PRECIPITIN TESTS IN EXPERIMENTAL AMOEBIC DYSENTERY IN CATS

Number of cats tested	Number of days since first appearance of amoebae in stools	Results of precipitin tests with amoebic antigen	Results of precipitin tests with bacterial control	Results
7	8 days or more	Serum undiluted +++ 1–1 +++ 1–2 +++ 1–4 ++ 1–8 –	Serum undiluted – 1–1 – 1–2 – 1–4 – 1–8 –	Strongly positive
3	6 to 8 days	Serum undiluted ++ 1–1 – 1–2 – 1–4 – 1–8 –	Serum undiluted – 1–1 – 1–2 – 1–4 – 1–8 –	Weakly positive
6	Less than 6 days	Negative in all dilutions	Negative in all dilutions	Negative
5	Normal	Negative in all dilutions	Negative in all dilutions	Negative
1	15 days—no lesions	Negative in all dilutions	Negative in all dilutions	Negative
2	Chronic—40 days	Serum undiluted ++ 1–1 + 1–2 – 1–4 – 1–8 –	Serum negative in all dilutions	Weakly positive
1	Recovered case of amoebae present 5 days then negative 40 days	Negative in all dilutions	Negative in all dilutions	Negative
3	haemolysed—could	not read reactions		

with this extract in an attempt to absorb a problematical nonspecific factor was then centrifuged and tested with the amoebic antigen. Since serums upon which such absorption tests were made did not differ from untreated serum in their reactions when in contact with amoebic antigen, we conclude that the reaction obtained with the amoebic antigen is specific.

A specimen of human serum obtained from a severe case of typhoid which gave a positive Widal in a 1–350 dilution gave a negative precipitin test with both amoebic and control antigens. The fact that these control tests have all been negative would seem to indicate that precipitins were produced by infected cats against the amoebae themselves, rather than against the by-products of ulceration, or against the secondary invading bacteria.

SUMMARY

1. Cats were successfully infected rectally with *Endamoeba dysenteriae* obtained from an acute human infection.

2. Amoebiasis in adult cats ran a more chronic course than in kittens. The virulence of the strain did not appear to be affected by passage from cat to cat.

3. Cats infected for a week or longer after amoebae were observed in the stools gave a positive precipitin test with an antigen prepared from scrapings of the ulcerated areas.

4. The serum from normal cats, or from cats infected for less than a week, gave negative precipitin tests.

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Transmitted December 12, 1923.

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EXPLANATION OF PLATE

PLATE 2

The precipitin reaction in cases of amoebic dysentery in cats.

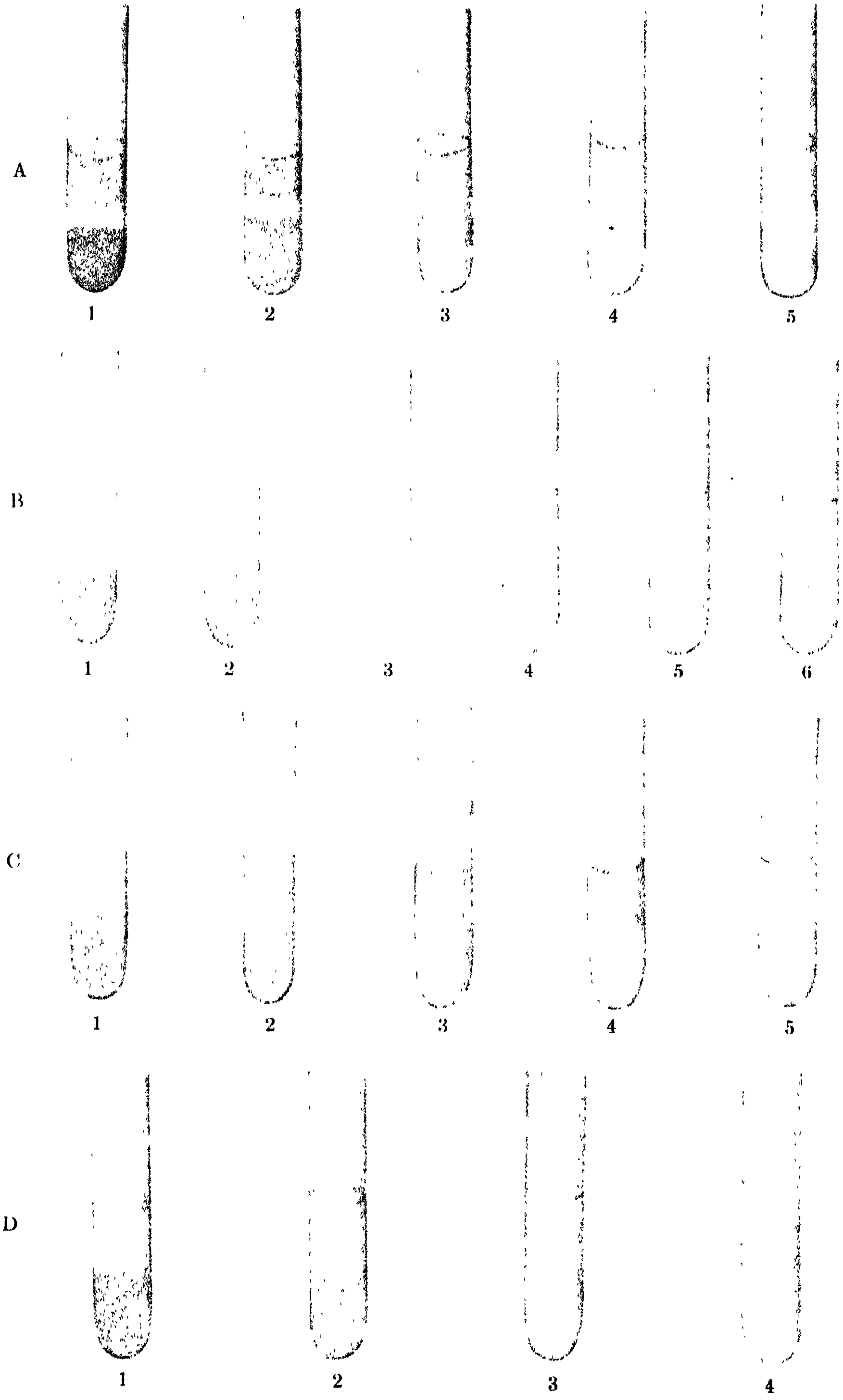
All drawings made after incubation of tubes sixteen hours in the ice box. Drawings by Dr. Olive Swezy.

Fig. A. Positive precipitin with amoebic antigen and the serum from a cat which had been infected with amoebae for sixteen days. Tube 1, undiluted serum; tube 2, serum diluted 1 to 1; tube 3, serum diluted 1 to 2; tube 4, serum diluted 1 to 4; tube 5, saline control.

Fig. B. Serum from the same cat as in figure A, but with bacterial control to the antigen. The dilutions of the serum are the same. Tube 5, serum from the same cat onto which the extract from the normal cat's intestine has been layered; tube 6, serum from a normal cat onto which extract from the normal cat's intestine has been layered.

Fig. C. Dilutions of serum from a normal cat onto which the amoebic antigen has been layered. Tube 5 is the saline control.

Fig. D. Dilutions of serum from a normal cat onto which the bacterial control to the antigen has been layered.


A
1
2
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B
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C
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D
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4

ON THE DISTINCTIONS BETWEEN ENDAMOEBA COLI AND COUNCILMANIA LAFLEURI

BY

CHARLES A. KOFOID, OLIVE SWEZY, AND JOHN F. KESSEL

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ON THE DISTINCTIONS BETWEEN ENDAMOEBA COLI AND COUNCILMANIA LAFLEURI

BY

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The senior authors recently (Kofoid and Swezy, 1921) described as new an amoeba from human stools which they designated as *Councilmania lafleuri.* This amoeba resembles *Endamoeba coli* in having an eight-nucleate cyst. It differs from that species in that it buds in the bowel, has clear instead of granular pseudopodia, and has eight instead of six chromosomes. In our opinion the two amoebae have, in the past, been confused and grouped together as *Endamoeba coli,* because each possessed an eight-nucleate cyst. Certain other workers, sometimes without apparent evidence of a critical reëxamination of material, have considered the two species as identical. Wenyon in his review in the Tropical Diseases Bulletin (vol. 19, pp. 19–22, 1922) concludes that "there is no alternative but to add the name *Councilmania lafleuri* to the long list of synonyms which *Entamoeba coli* already possesses."

The effect of this pronouncement in a journal of the standing of the Bulletin is reflected in the acceptance of this conclusion in certain textbooks such as those of Brumpt (ed. 3. 1922) and of Nöller (1922) and in reviewing journals such as the Bulletin of the Pasteur Institute (Paris) (vol. 20, p. 141, 1922), and by an anonymous reviewer in Nature (London, 1922).

It is the purpose of this paper to present some additional evidence, on the basis of which we reaffirm our conclusions in the earlier paper, to meet, as far as may be, the criticisms advanced by Wenyon, and to point out the rather far-reaching consequences of our findings on methods and practice in diagnosis of amoebiasis. The new evidence we offer is in part an extension of previous lines of observation and in part arises from our experimental infections of culture rats and mice.

In order that the reader may appreciate the extent of the material bearing on this matter which has been available to us, we quote the following statistics from our unpublished data from the Parasitological Laboratory of the California State Board of Health.

In the interval between February 16, 1921, when we first began to make the distinctions between *E. coli* and *C. lafleuri*, and November 9, 1923, we have made 20,264 stool examinations on 4763 persons, an average of 4.25 per person, including a number of reëxaminations after treatment. In this group of persons, mostly patients of physicians, we found 181 positives for *C. lafleuri* without accompanying *E. coli*, or 3.8 per cent, and 192 accompanying that amoeba, a total of 373 or 7.83 per cent. We also found 349 cases of *E. coli* without *C. lafleuri* or 7.32 per cent. If the 192 infections of *E. coli* with *C. lafleuri* are added, the total for *E. coli* rises to 541 or 11.35 per cent.

Since June 30, 1922, we have made from 300 to 800 stool examinations per month. The monthly percentages are not significantly different from those for the longer period. The material available has consisted of stools from patients in the University of California Infirmary, and of those sent in by messenger, mail, and express from various parts of California and from many other states. It has included a considerable number of fresh stools in warm thermos bottles for examination of the living amoebae, both with and without antecedent saline purge. In addition to hundreds of slides made especially for observation on these two species, we follow the custom of fixing and staining all doubtful cysts and all positives for *Endamoeba dysenteriae*, with the result that we have not lacked either for experience in distinguishing the two species or for varied and abundant material.

Wenyon's statement that "it would be quite impossible in ordinary work to distinguish either the vegetative stages or the cysts of the supposed *Councilmania lafleuri* from those of *E. coli*" is not borne out by the practical experience of ourselves and of Miss Inez Smith, our regular technician, and of more than thirty-five different investigators, technicians, physicians, and graduate students working in our laboratory since January, 1921. The experience of the senior author in teaching these distinctions to students in three classes in protozoology, totaling 105 persons, makes us sanguine that the average student can learn to make the distinction so as to diagnose the infections by means of the cysts.

Wenyon's further statement that "it can serve no practical nor scientific purpose to regard the forms described as belonging to a distinct species" is true only if his surmise is correct and we have, as he supposes, mistaken the motile stages of *E. dysenteriae* for those of our *Councilmania,* and the supposed cysts of the latter are really only those of *E. coli.*

The practical feature of the matter lies in the widespread custom in the past, and even persisting to the present day, for manuals of clinical medicine to designate the presence of clear pseudopodia in the motile phase of an intestinal amoeba to be diagnostic of *E. dysenteriae* as over against the supposedly harmless *E. coli.* A continuation of this practice which might follow from Wenyon's statement will inevitably lead to false diagnoses in those cases where only *Councilmania lafleuri* but not *E. dysenteriae* is present. This, it seems to us, is a matter of scientific accuracy and of very great practical import.

Wenyon's surmise that *E. dysenteriae* was in reality present when we found motile amoebae with clear pseudopodia associated with budding cysts of *Councilmania* seems to us to have little weight for the following reasons:

1. In routine stool examinations six consecutive negative findings are customarily interpreted only as a probable negative for *E. dysenteriae.* It has been computed by Carter, MacKinnon, Matthews and Smith (1917) on a statistical basis that, on three examinations, 64.6 per cent of the infections ultimately found were detected, while after five the percentage rises to 90.4 per cent, assuming that the sixth examination yields 100 per cent. Our frequent use of the saline purge and our routine practice of making six examinations whenever feasible, and more if clinical signs of amoebiasis are present, at least maintain this ratio of probability of accuracy in our findings. Thus in very many of our cases in which *E. dysenteriae* is excluded as a concomitant with *C. lafleuri,* we have made six examinations and in some instances many more.

2. Pure infections with *C. lafleuri,* i.e., without cysts of *E. dysenteriae,* invariably yield motile forms with clear pseudopodia when tested.

3. Mixed infections with cysts of *E. dysenteriae* and *C. lafleuri* and without *E. coli* do not yield when tested any motile forms with granular pseudopodia, as they should if the cysts of *C. lafleuri* are referable to *E. coli,* and its motile stages to *E. dysenteriae,* as Wenyon surmises.

4. Rats experimentally infected with stools containing only *C. lafleuri* and no *E. dysenteriae* yield cysts and motile forms of the former only. No cysts of *E. dysenteriae* are developed.

5. Clear pseudopodia are not produced in motile forms of *E. coli* by the saline purge as Nöller (1922) suggests they may be. The

pseudopodia of *Councilmania* are not changed as to hyalinity by the presence or absence of a saline medium created by the purge. Neither are those of *E. coli* changed.

In view of these facts and considerations, we reaffirm our original conclusion that *Councilmania lafleuri* has clear pseudopodia; and we further state that Wenyon's surmise that the motile forms attributed by us to *C. lafleuri* are really to be attributed to *E. dysenteriae*, cannot, in our judgment, be correct.

Data from two cases of *C. lafleuri* in man are here presented. The first case, and the one upon which our original investigation was made, was that of a soldier returned from France with chronic amoebic dysentery. He was examined by us, February 3 to 10, 1920, and shortly afterwards treated at the University Infirmary by the emetin-bismuth-iodide method. The treatment was followed by clinical recovery. The stools also contained *Chilomastix davainei*, *Blastocystis hominis*, and yeasts. Between April 13, 1920, and November 1, 1922, examinations were made of 88 additional stools, from a number of which numerous slides, about 700 in all, were made, without the discovery of any infection by *E. dysenteriae* in any phase.

On February 1, 1921, after 25 examinations subsequent to treatment for *E. dysenteriae*, *Councilmania lafleuri* appeared in the stools and continued until May 5, being found in 54 of the 58 stools examined in that period.

On May 16 it disappeared entirely, after a cumulative treatment with antimony trioxide. Marked indications of nuclear disintegration were detected in the cysts prior to their final disappearance. Between May 16 and November 7, 1922, the stools were negative for *Councilmania* on all of 30 examinations.

During the entire 119 examinations, no cysts of *E. coli* were found and no motile amoebae with granular pseudopodia were observed. The absence of cysts of *E. dysenteriae* in the 113 stools suggests that *E. dysenteriae* had been exterminated by treatment. From these stools far more than the usual number of slides were made and searched with more than the usual thoroughness in the course of our work on *Councilmania* in this case.

If Wenyon's surmises are correct, this case must have had during this period a mixed infection of *E. coli* and *E. dysenteriae*. and after treatment the case was not cured of the infection, although the clinical signs of recovery were striking. Furthermore, if he be correct, we must have seen only motile forms of *E. dysenteriae*, and these also in great abundance, while no cysts of it were found. We must also have seen cysts of *E. coli* in abundance, but none of its motile stages. The only motile amoebae seen by us in this case in this period after treatment were, aside from a few *Endolimax nana*, the large, active amoebae some with ingested red blood corpuscles, with clear pseudopodia which invariably characterize infections of *Councilmania lafleuri* whenever we can secure motile amoebae from human stools having *C. lafleuri* cysts.

We therefore conclude that this was originally a case of infection of *E. dysenteriae* which was cured, and that the motile amoebae subsequently observed belonged to the same species as did the cysts occurring in that period. If this species is *E. coli*, then *it* had clear pseudopodia under all conditions of their appearance in the course of laboratory examination. Our interpretation is that neither the cysts nor the motile forms belong to *E. coli*, nor do the motile forms belong to *E. dysenteriae*. Both cysts and motile forms belong to the species we have characterized as *Councilmania lafleuri*.

A second case, which is of the type often represented in our series of cases, is one in which there has been no finding of *E. dysenteriae*. This case is that of Mrs. X, age 57, with severe arthritis. Between June 1, 1921, and June 2, 1923, we made 72 stool examinations and often used the material for numerous slides for study. The examinations have usually been made on groups of two to six successive stools, and have been made repeatedly on carefully fixed and stained smears.

This case carried infections of both *E. coli* and *C. lafleuri* as well as *Endolimax nana* and *Blastocystis*. It exhibited motile amoebae of both types, those with clear pseudopodia and those with granular ones. *Councilmania lafleuri* was detected in 32 of the 72 stools. After a series of several cumulative treatments with antimony trioxide, this amoeba disappeared from the stools in the period from November 22, 1922, through the last of six examinations, on June 2, 1923, and on a later run of six consecutive specimens from October 29 to November 3, 1923, a total of 12. *Endamoeba coli* was detected in 23 of the 72 stools and continued after the disappearance of *Councilmania* in two of the twelve examinations in that period.

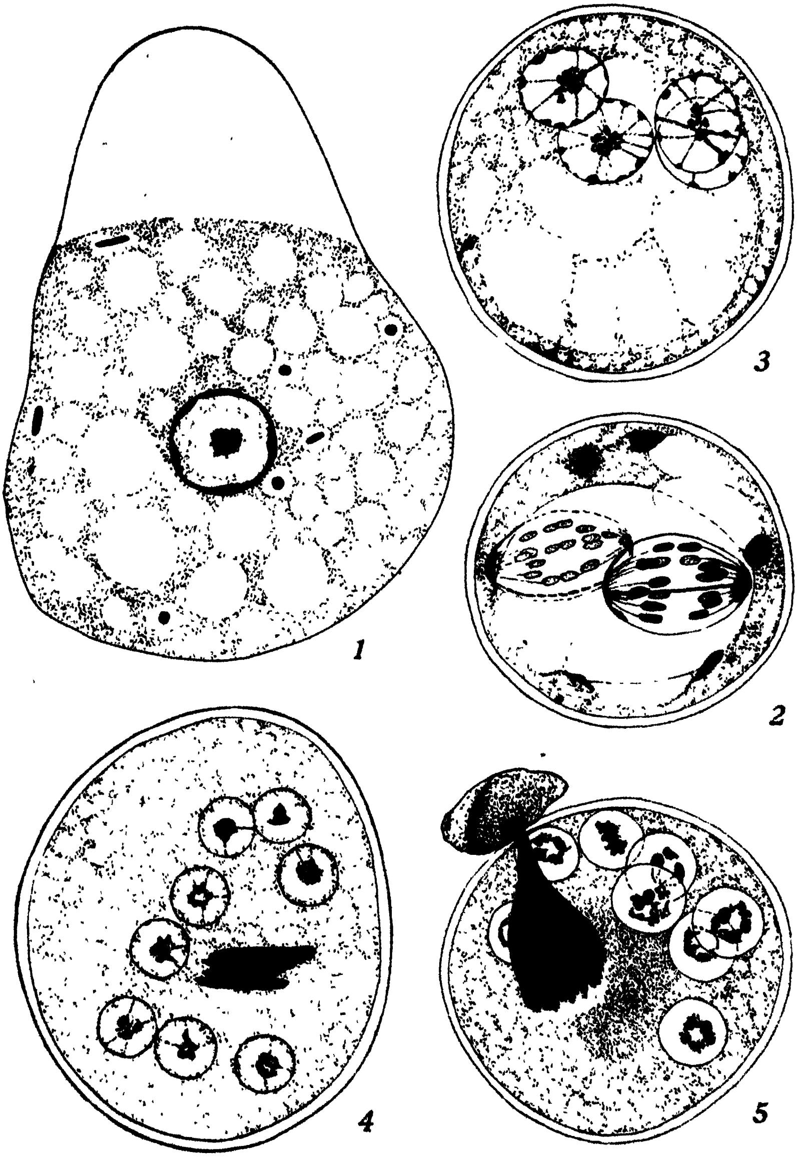
In the case of Mrs. X, *E. dysenteriae* was never detected in 72 examinations extending over nearly three years, yet motile amoebae with clear pseudopodia of the *Councilmania* type were present therein. In the first case, on the other hand, no motile amoebae with granular pseudopodia were ever seen by us in the stools and no cysts of *E. coli*.

Other cases of similar import have occurred in our examinations, but these two are cases in which our examinations were most numerous, most extended in time, and most thorough.

Our data on these cases are subject to the restriction that no negative result is ever theoretically conclusive. The order of magnitude of our observations in these cases, however, is such as to increase the probability of the correctness of the conclusions we draw from the data. In the case of different species, the probability of a negative result being correct is different, depending upon the relative abundance, which appears to be somewhat of a species characteristic. Thus, in our experience, there is a difference in this respect between *E. dysenteriae* and *E. coli.* The former, except in old chronic cases, often with arthritis in which the amoebic cysts of the bowel are small and usually few, is subject to recurrent periods of considerable abundance. *E. coli*, on the other hand, is relatively less abundant and for this reason is perhaps more often brought to light by prolonged series of examinations than are other species. In our experience in over 30,000 examinations, it is also apparent that mixed infections are more frequent than single ones and that numerous examinations over long periods sometimes reveal a hitherto undetected infection.

Budding.—In so far as we can ascertain, no one, with the possible exception of Casagrandi and Barbagallo (1897), has, previously to our account, described budding or the emergence from its cyst of a parasitic amoeba of man. The Italian investigators observed it in cysts of human amoebae in the cat. It is uncertain with what species they were dealing. The possibility that it might be *C. lafleuri* is not precluded by their data. There is no record in any work known to us of budding in the cysts of *Endamoeba dysenteriae, Endolimax nana*, or *Iodamoeba bütschlii*, or in those of *Dientamoeba fragilis,* in the stools in which they are found, or anywhere else, although the first-named species has been much under observation. As to *E. coli* in our sense, we have never seen the cysts budding in the stools in which they are formed, although we have searched our material intently for it. We do find this process in *C. lafleuri* in the stools in which its cysts are formed. This does not occur in all stools, nor in equal degrees and frequency, but it does recur again and again in the same case, and search may bring it to light where it is seemingly absent.

We use the term budding advisedly, since it is clearly a biological process and is the successive detachment, through a definite pore, of amoebulae, each with a single nucleus. We have found them in all phases of emergence and have watched one, on a freshly made slide, emerge, detach itself, and move away. We have found detached amoebulae near the pore and near the budding cysts. The same type



Figs. 1–5. *Councilmania lafleuri* from human intestine. × 3500. Fig. 1. Active form showing clear pseudopodia. Fig. 2. Binucleate cyst with nuclei in anaphase showing 8 (16) chromosomes and intradesmose. Fig. 3. Four-nucleate cyst with nuclei showing prophase of third division. Fig. 4. Eight-nucleate stage, with typical nuclei with large diffuse karyosome. Fig. 5. Bud forming with chromatoidal contributing to outpouring cytoplasm.

of process of budding takes place in two species of amoebae which belong to *Councilmania* found in the rat and mouse (Kessel, 1923).

Wenyon, in criticism of our interpretation, says:

> They have produced, however, no evidence that the escape of the supposed new amoeba from the cyst is different from that of *E. coli* from its cyst. The only variation is that they claim that the process takes place in the host in which the cysts are produced instead of in the next host. They draw attention to a figure of the cyst of *E. muris* of the mouse published by the reviewer in 1907, who thought at the time that this might be a developmental process but now realizes that he was dealing with a ruptured cyst.

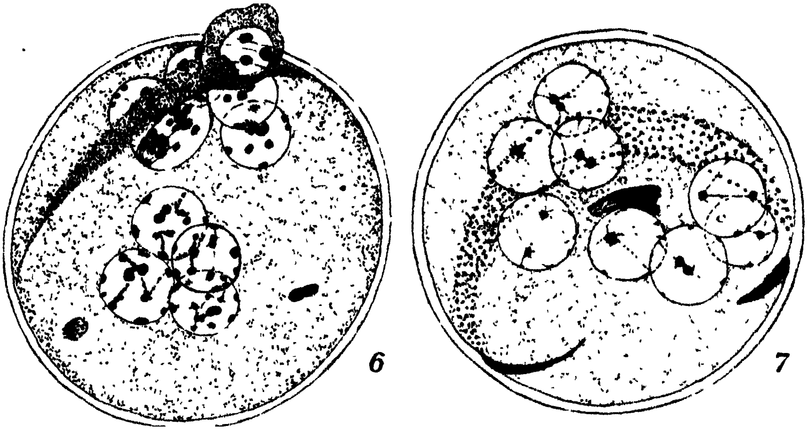
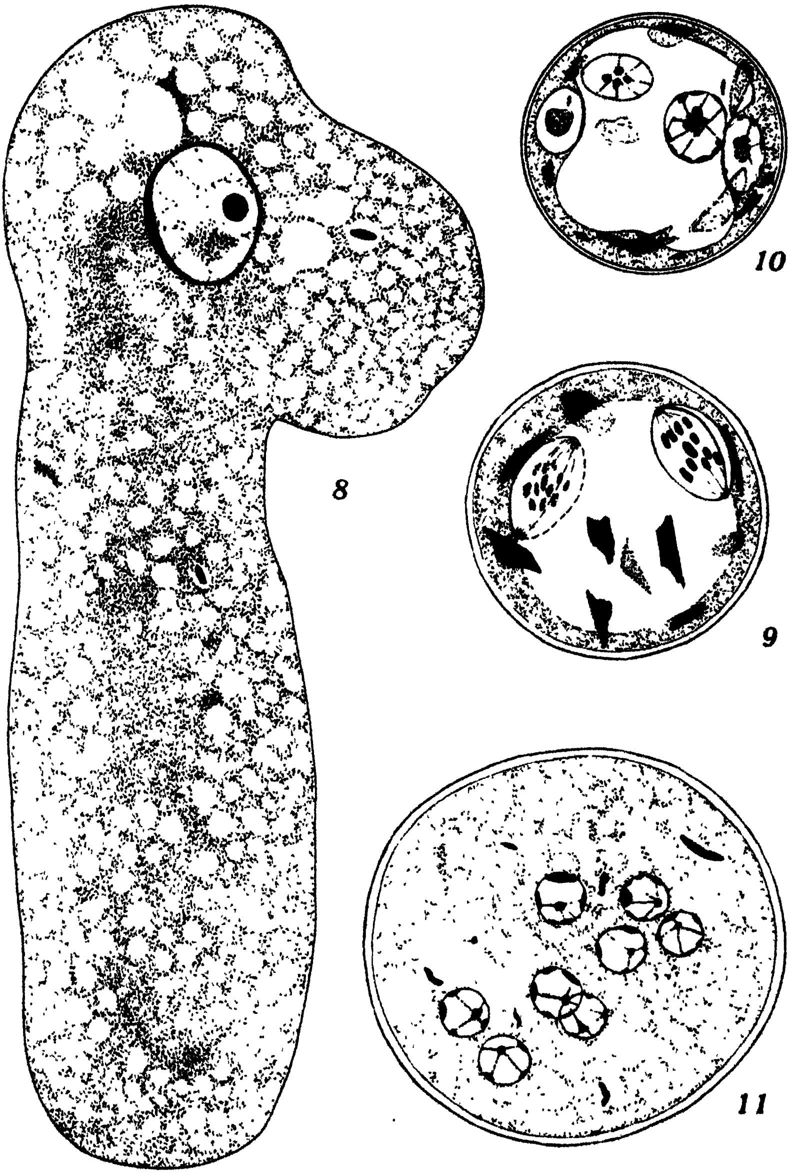


Fig. 6–7. *Councilmania lafleuri* from human intestine. × 2500. Fig. 6. Cyst with bud containing single nucleus just escaped from the cyst. The remaining nuclei show prophase stages with the exception of one near the top which is in early anaphase with 8 chromosomes. Note chromophile ridge of dense, heavily staining cytoplasm. Fig. 7. Nuclei in early prophase. Chromophile ridge in lower surface of cytoplasm.

Our critic is correct in noting our "claim" that the process takes place in the host in which the cysts are produced. The difference from *E. coli* lies in the fact that this process does not, so far as we can ascertain, take place in this species in the host in which the cysts are formed. The evidence we present in support of this is that we have observed buds in hundreds of cysts of *C. lafleuri* in single and mixed infections, but never in any other species, including *E. coli*, associated with *C. lafleuri* in the stool and subjected to similar treatment. Buds have been found at autopsy *in the caecum* of the rat on cysts of *C. lafleuri* experimentally introduced, and on cysts of *C. muris* and *C. decumani* in the caecum or in stools of the culture rat. We have found them in fresh human stools from warm thermos bottles within a short time after defaecation. Not knowing ourselves just how and when *E. coli* does escape from its cyst, we are of course unable to state in what respects the budding processes of the two amoebae differ save that we cannot find *E. coli* budding in the stool and we do find buds on *C. lafleuri.* It is possible that the typical digestive sequence in hydrogen ion con-



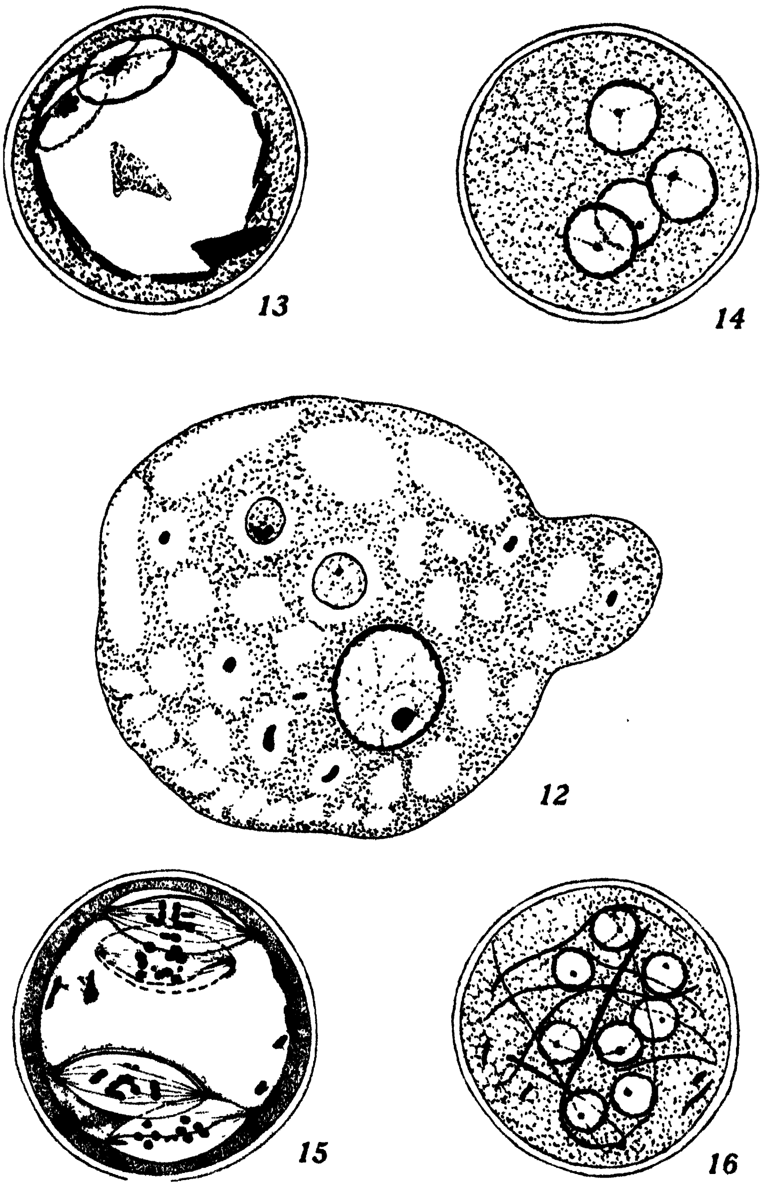
Figs. 8–11. *Endamoeba coli* from human intestine. × 2500. **Fig. 8.** Active form with long, granular pseudopodium. **Fig. 9.** Binucleate cyst with splinter-like chromatoidals and two nuclei in early anaphase, each with 6 (12) chromosomes and intradesmose. **Fig. 10.** Four-nucleate cyst. **Fig. 11.** Eight-nucleate cyst showing typical nuclei with small excentric karysome and blobs of chromatin on nuclear membrane.

centration, if it should occur at the focus of infection by *E. coli*, might under exceptional circumstances induce budding in that species while still in the bowel, but we have been unable to find such budding cysts either in fresh stools or in those of various ages.

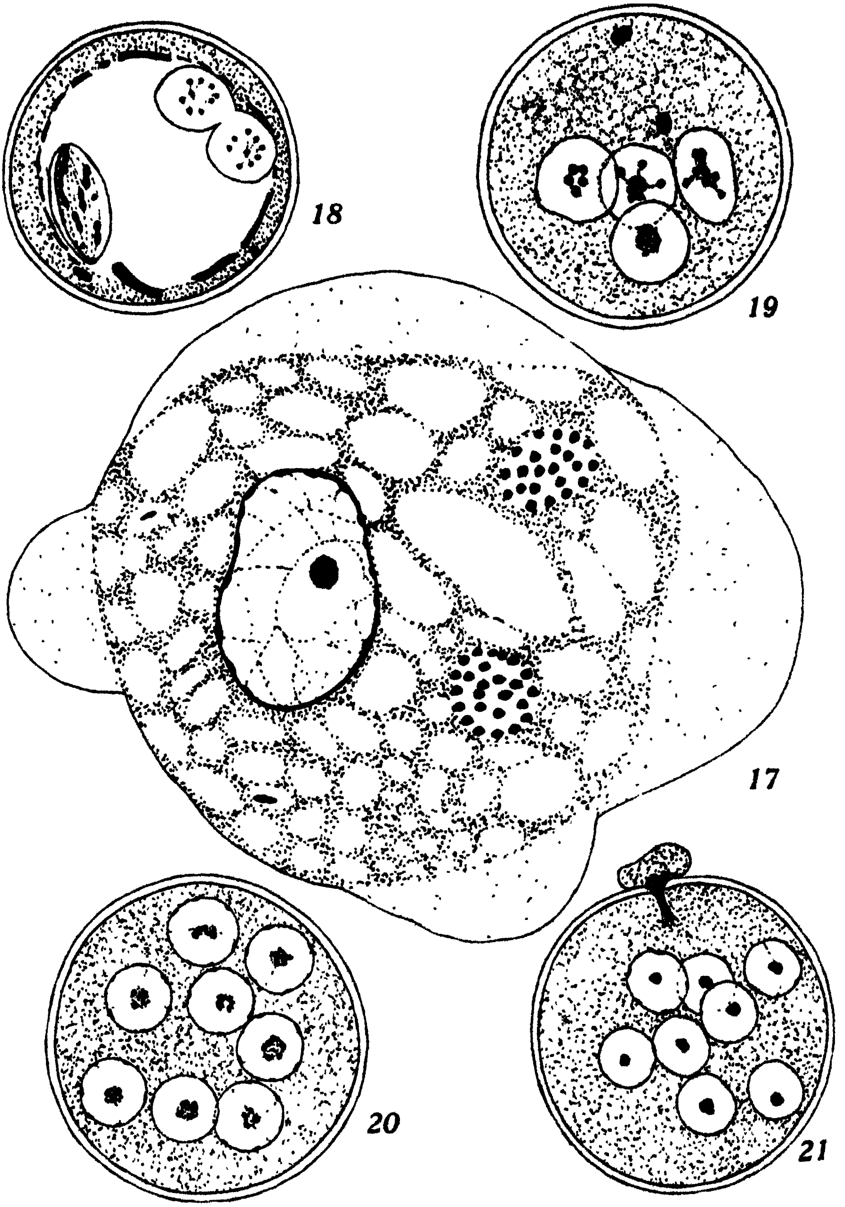
Wenyon also raises the objection that the "buds" are artifacts, stating

> In the very numerous observations the reviewer has made on cysts of *E. coli* he has noted on many occasions that in saline preparations it is quite easy to rupture the cysts of *E. coli* between the slide and cover glass. In such cases the cytoplasm protrudes as a hernia, and this may contain a single nucleus, while the other nuclei are congregated about the opening in exactly the manner described by Kofoid and Swezy for their new amoeba and depicted in their figs. 22 and 27. . . . The authors have considered the possibility of the appearance being produced by a rupture of the cyst, but they put this aside with the statement that "it is not due to trauma and cannot be produced by pressure." From the observations on cysts of *E. coli* mentioned above the reviewer is in a position to state that appearances of the same kind can certainly be produced by artificial rupture of the cyst of *E. coli.*

We have tested this hypothesis repeatedly by experiment and our results do not agree with Wenyon's, who significantly does not state that he bases his conclusions on new experiments directed to this end; it would appear that his remarks may be based on current interpretations of earlier observations. Our alternative suggestion is that what he really observed were budding cysts of *C. lafleuri*, whose buds Wenyon now concludes were produced by "rupture between the slide and the cover-glass." While it is true, doubtless, that cysts can be compressed between slide and cover, the rupture of a non-budding cyst in most stools is very difficult; it requires a very thin film of faeces and repeated efforts to effectively direct the pressure, provided the same cyst is kept in view throughout the process. When ruptured, the appearance is unlike the appearance of the bud, the rent (fig. 24) being unlike the pore, and the outpouring cytoplasm unlike the bud in size and form. If the pore is already present the cytoplasm could doubtless be squeezed out through it. If budding cysts are already present in the stool, one might easily conclude that pressure had produced them. Non-budding cysts kept constantly in view during pressure do not in our experience 'bud.' They can be crushed with difficulty, but they do not give the picture of budding. This picture, moreover, is characteristically, one of a normal biological process. It results in the occurrence of cysts in the stool with varying numbers of nuclei from sixteen down to one. Hence Wenyon's (1922, p. 22) statement that "the pore through which the buds are supposed to be



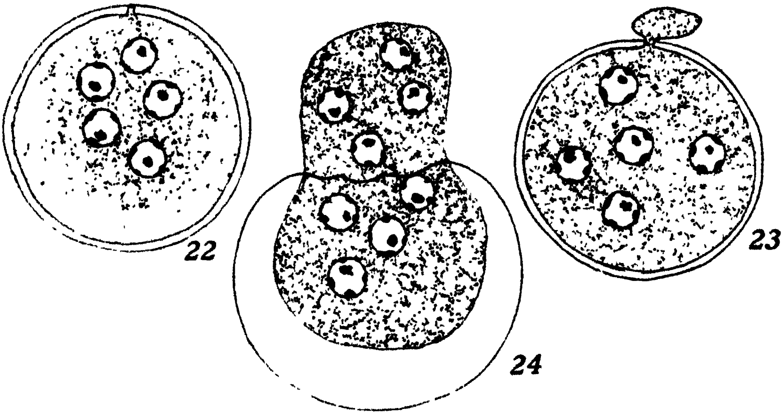
Figs. 12–16. *Endamoeba coli* from rat which had been fed human faeces containing *E. coli*. × 2500. Fig. 12. Active form with granular pseudopod. Fig. 13. Binucleate cyst with nuclei in early prophase. Fig. 14. Four-nucleate cyst with typical nuclei showing small excentric karyosome and chromatin on nuclear membrane. Fig. 15. Four-nucleate cyst with nuclei in early anaphase with 8 (16) chromosomes and intradesmose in each. Fig. 16. Eight-nucleate cyst showing typical nuclei with small excentric karyosome and encrusted membrane.



Figs. 17–21. *Councilmania lafleuri* from rat which had been fed human faeces containing *C. lafleuri* cysts. × 2500. Fig. 17. Active form with clear pseudopods. Fig. 18. Amoeba with three nuclei, two nuclei in late telophase showing centrosome and attached 8 chromosomes in each, while the other nucleus is in late prophase. Fig. 19. Four-nucleate cyst showing typical nuclei with diffuse karyosome. Fig. 20. Eight-nucleate cyst with typical nuclei. Fig. 21. Budding cyst.

formed is depicted as being already present in the four-nucleated stage'' is a different interpretation from ours. This four-nucleated stage resulted from the escape of four nuclei from the previously eight-nucleated cyst.

Moreover, the budding cysts are found in stools fixed while still warm, not only in smears made without pressure, but in stools fixed in hot Schaudinn's fluid and sectioned by the paraffin method. No traumatic pressure was applied during this process and the picture revealed by the budding cysts in the sections is identical in form with that in fresh and in fixed and stained smears.



Figs. 22–24. *Councilmania decumani* from rat faeces. × 2500. Fig. 22. Cyst from which several amoebulae have escaped, showing the pore with a core of cytoplasm filling it. Fig. 23. Cyst with the cytoplasm escaping as a bud. Note the size of pore in both cysts. Fig. 24. Cyst which has been ruptured, with large amount of cytoplasm protruding. Compare size and shape of opening with the pore in figures 22 and 23.

The chromophile ridges.—We described (1921) as the chromophile ridges a deeply staining, slender or broad, single or tripartite, peripherally located, protoplasmic structure often associated on the one hand with the pore and on the other with the centrally located bundle of chromatoidal bodies or their remnants (figs. 6–7). This chromophile structure seems to antedate the bud in appearance and has generally disappeared early in the budding phase. It persists in some cysts far along in that process.

These chromophile structures are without any shadow of doubt protoplasmic in composition. They are differentiations of the peripheral cytoplasm, possibly by incorporation of the dissolving substance of the chromatoidal body, or by migration of protoplasm containing this substance to the periphery. The tendency of the cytoplasm passing from this ridge into the pore to be more deeply stained suggests the condensation of the more stainable part of the cytoplasm in the ridge, pore, and at times in the bud. The chromophile structure is rarely more than very slightly raised (Kofoid and Swezy, 1921, pl. 21, fig. 21; pl. 22, figs. 25, 26) above the general level of the spherical contour of the cytoplasm (figs. 6–7), except as it is forming the pore or emerging from it.

Wenyon writes (1922, p. 22) that "the chromophile ridge on which they lay so much stress appears to be in some cases nothing more than a fold or crease of the inner membrane of the cyst, which is readily seen in ruptured cysts of *E. coli.*" Anyone can easily convince himself of the incredibility of this conclusion. In the first place, the cyst walls of amoebic cysts properly stained and destained, including every one of those figured in this and in our earlier paper, do not stain, nor do we find an inner layer ever differentiated *by its stainability.* Furthermore, in both the cysts and our figures, which are accurately drawn, the ridges are *in* the cytoplasm, and the cyst wall retains its normal contour. It is not wrinkled. His further statement that "in others it possibly represents chromatoid bodies" is demonstrably incorrect, as may be proved by inspection of any and all properly stained cysts. The ridge is composed of granular or alveolar cytoplasm which is very definitely shown in heavily decolorized cysts (fig. 6). The chromatoidal bodies are homogeneous, and are not easily confused with the ridges. Furthermore, to us these chromophile structures seem to have the semblances of morphological structures associated with a process in which the metabolic changes of the chromatoidal bodies are intricately involved. They clearly foreshadow the phenomenon of budding and are associated with the location of the pore and with the more deeply staining nature of the cytoplasm of many of the buds. Cysts do occur, however, with buds, generally in late stages of budding, in which neither chromophile substance nor chromatoidal bodies are present at the time.

Pseudopodia.—The pseudopodia of *Councilmania lafleuri* are entirely different from those of *E. coli.* We have observed them in both species in stools of both supposedly single and mixed infections after transfer to culture rats (Kessel, 1923). In both the former and the latter, we have had material in which *E. dysenteriae* had never been found.

In *C. lafleuri,* the living pseudopodia are remarkably clear, broadly rounded in contour, their length rarely equal to or exceeding their width at the base, which often exceeds half the diameter of the body. They taper but little and their clear ectoplasm is sharply delimited from the adjacent granular endoplasm (figs. 1, 17).

They are usually formed with explosive suddenness in normal motile individuals and tend to linger without change for longer or shorter periods. A second, and rarely a third pseudopodium may be formed before the first is invaded by endoplasm or withdrawn. In chilled or moribund individuals, the pseudopodia are smaller, less extended, and sluggish. There is little or no evidence of any persistent polarization in locomotion. We find no evidence of attachment to the substrate.

In *Endamoeba coli* the pseudopodia are granular, being filled with endoplasm as they form (figs. 8, 12). They are generally narrower, more tapering, more variable, and give somewhat more evidence of polarization in locomotion, that is, the pseudopodia form at or near the anterior end of the somewhat elongated body about equally on the two sides of the axis of progression, perhaps spirally (Schaeffer, 1920). This polarization is periodically interrupted and perhaps readjusted by a sort of motor reacton. This amoeba often attaches itself to the substrate and at such times exhibits a small adhesive cone at its posterior end.

Both amoebae may be filled with food vacuoles, but we have thus far seen red blood corpuscles in *Councilmania* only. It may, of course, be possible that *E. coli* will ingest them; *Councilmania* certainly does to a considerable degree. The food vacuoles in both (figs. 1, 8, 17) may be elongated, especially when containing bacteria.

The nuclei of the cysts.—The nuclei in the encysted stages afford one of the most useful and critical criteria for distinguishing between infections by the two amoebae. This criterion is, however, one requiring the appreciation of cytological detail, good preparations, and the best optical equipment. To interpret all stages one must appreciate the changes attending mitosis, the shifting of the central karyosome to the periphery, the emergence of the split chromosomes on the nuclear membrane during and subsequent to this process, their organization in the more or less dispersed equatorial plate, and their disappearance with the formation of the karyosomes of the daughter nuclei. The earlier part of the process is more prolonged and gives rise to variations in the location of the karyosome and to degrees of its dispersion in granules which appear to contribute to the substance of the chromosomes.

Good preparations are essential. Our best results are obtained by fixation in Schaudinn's fluid with 4 per cent glacial acetic acid added before using. This fixer is used preferably at 60° C. on fresh, undried smears. The sublimate is removed by iodine-alcohol. Mordanting is done in 2 per cent iron alum and staining in 0.5 per cent aqueous haematoxylin made from tested. white crystals. The mordant and stain are used at 30° C. We do not find that smears prepared by the Mayer's haemalum method recommended by Dobell are satisfactory, as they lack the sharp detail which the iron haematoxylin provides. Our optical equipment includes monobjective binoculars of Bausch & Lomb, E. Leitz, Spencer Lens Company, and Carl Zeiss, with apochromatic and fluorite objectives and compensating eyepieces, and the new McClung model of the Spencer Lens Company with special substage with oil between slide and condenser and a 400-watt Mazda lamp with daylite glass for illumination. These details are given in view of Wenyon's statement of his grounds for not accepting our statements as to nuclear structure and chromosomes.

Wenyon comments (1922, p. 22) in his review,

> The assertion that the new amoeba has eight chromosomes during nuclear division, whereas *E. coli* has only six, comes as a surprise to the reviewer, who was under the impression that no definite chromosomes are formed during the division of the nucleus of *E. coli*. Such stages of the division of the nucleus of *E. coli* as he has seen give no indication of chromosome formation, and this is borne out by Dobell's detailed description of the division of the nucleus of *E. histolytica*, which is of a similar type.

It would have been fairer to the readers of the Tropical Diseases Bulletin and more just to our work had the reviewer not restricted his presentation of our evidence to the word "assertion." We clearly showed (1921, pl. 20, fig. 13) two nuclei drawn with greatest fidelity to the material, with 8 (16 at early anaphase) chromosomes. We also showed many other stages at the mitotic period (our plates 20 and 21) and much other evidence is in hand. This in all fairness is more than an "assertion." In like tenor A. A.[lcock] (1923) in a brief review (Trop. Dis. Bull., vol. 20, p. 707) of Swezy's (1922) account of mitosis in *Endamoeba coli* writes "*Entamoeba coli* in mitosis is said to possess six chromosomes," whereas evidence of the existence of chromosomes is depicted in carefully made drawings of at least nine cysts and eighteen nuclei. Some of these at the critical period of the early anaphase show distinctly the evidence that the normal number of chromosomes in *E. coli* is six.

The fact that Dr. Wenyon and others may not have seen chromosomes in *E. coli* or other amoebae of man is readily understood by all who hunt for them. One must *find the cysts in the right stages* and these are rare and are evidently of short duration. Doubtless Dr. Wenyon would be the last to insist that our results are invalid because he or anyone else had not seen the chromosomes, or found them in late anaphases of dividing nuclei in active amoebae in the kitten.

In another particular we reaffirm the distinction between the two amoebae in question, namely, the structure of the karyosome. See in this connection Kofoid and Swezy's (1921) figures of *C. lafleuri* and Swezy's (1922) of *E. coli.* See also in this paper figures 1 to 7, and 17 to 21 of *C. lafleuri* and figures 8 to 11 and 12 to 16 of *E. coli.* Wenyon wavers somewhat in his attitude on this point, admitting that "the nucleus [of *C. lafleuri*] is certainly different from that of typical *E. coli* or *E. histolytica,* but it is well known, though perhaps not sufficiently recognized, that both these amoebae frequently show nuclei which are far from typical. The reviewer has often seen the appearances here depicted in undoubted *E. coli* or *E. histolytica.*" This statement is true thus far: that nuclei far from the so-called typical stage, presumably the resting stage in the cyst, are to be found. Some of these are attendant upon mitosis and have been the subject of intensive study on our part. Other modifications are probably due to a pathological or a moribund state of the amoeba. Our figures of *Councilmania* are not of the latter type. With the inference which one might make from Wenyon's sweeping statement that he "has often seen the appearances here depicted in undoubted *E. coli* or *E. histolytica*" we are not in agreement. If he saw dispersed chromatin masses in these two species, they were phases of mitosis. If he saw in resting nuclei the type we figure in the resting phases of nuclei in the cysts of *C. lafleuri,* it is our opinion that he had *C. lafleuri* under his eyes.

The point to be particularly noted in this connection is the fact that the nuclei of *C. lafleuri* habitually, consistently, and predominantly show karyosomes of the dispersed type shown in our figures. They are central rather than excentric, larger and more dispersed, with the constituent granules more or less discrete and distinct (figs. 3, 4, 5), or, if massed in one granule (figs. 1, 21), this granule has a central location and shows peripheral lobing It is larger and less spheroidal than in *E. coli,* and its massed phase is very rarely seen.

SUMMARY

The conclusion of Wenyon's review is that "the amoeba *C. lafleuri* is none other than *Entamoeba coli.*" The following facts controvert this conclusion:

1. The case reported in our first paper had been clinically cured of an infection by *E. dysenteriae* and thereafter had motile amoebae with clear pseudopodia and cysts of *Councilmania* but none of *E. dysenteriae* in 113 examinations.

2. Cases in which only *C. lafleuri* was detected exhibit motile stages with clear pseudopodia only, while some of the attendant cysts appear with chromophile ridges, pores, buds, variable numbers of nuclei whose central karyosomes are dispersed, and with eight chromosomes at mitosis. These characteristics are persistent in cases of infection continuously examined.

3. Cases in which only *E. coli* was detected present motile stages with granular pseudopodia only, and the attendant cysts do not have chromophile ridges, pores, or buds. The karyosomes are excentric, small and solid, and the chromosomes are six in number. These characteristics are persistent when tested.

4. Mixed infections present both types.

5. These characteristics remain as stated on transfer to rats in cases of single and mixed infections.

Transmitted November 20, 1923.

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THE BORING MECHANISM OF TEREDO

BY

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THE BORING MECHANISM OF TEREDO

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INTRODUCTION

The destructive habits of the wood-boring mollusks of the family Teredinidae have been the occasion of much scientific interest as well as popular concern. Following a period of pelagic life, the minute bivalve larvae of these organisms, such as find lodgment on a suitable piece of submerged timber, speedily bury themselves in its water-macerated surface. They become permanently attached by a fleshy collar near the point of entrance, and elongate inward, boring as they grow, until in certain species a length of more than three feet is attained, and a diameter at the anterior end of upward of an inch. By reason of their immense numbers and rapid growth, they speedily reduce unprotected wharf piling and ships' timbers exposed to their ravages to mere honeycombed masses of débris.

The method by which this destructive work is accomplished has been a subject of much discussion. The animals will not bore again if removed from the wood; and if an opening be made into the burrow from without, they will repair the breach with a nacreous wall before they recommence boring. Attempts to observe the boring process have uniformly failed, and the inferences drawn from indirect evidence present much confusion and contradiction. Hence it comes that after several centuries of speculation, experiment, and debate, the actual method of boring has remained in doubt.

ACKNOWLEDGMENTS

The investigation here reported was undertaken at the suggestion and under the direction of Professor Charles Atwood Kofoid, whose advice and assistance I gratefully acknowledge.

The coöperation of the U. S. Lighthouse Service, the U. S. Navy Yard at Mare Island, the Board of State Harbor Commissioners, the Southern Pacific Company, and the California and Hawaiian Sugar Refining Corporation, in placing test boards at various localities in San Francisco Bay, has made possible the securing of the specimens of *Teredo navalis* and *Bankia setacea* used in this study. The *Teredo diegensis* material was collected by the writer in San Diego Bay, through the coöperation of the Public Works Department of the U. S. Naval Headquarters at that place. The specimens of *Teredo affinis* and *Bankia mexicana* were taken from test boards planted at Nawiliwili, Hawaii, and Mazatlan, Mexico, respectively, under the auspices of the National Research Council Committee on Marine Piling Investigations.

The work has been carred on as a phase of the investigations of the San Francisco Bay Marine Piling Committee, to whose constant interest and support this investigation is primarily due.

REVIEW OF LITERATURE

The first writer to consider at all critically the problem of the method of boring of *Teredo* appears to have been the Dutch investigator Sellius, whose scholarly "Historia Naturalis Teredinis" (1733) is a classic of the early literature on marine borers. Sellius maintains, after reviewing the opinions and conjectures of his predecessors, that the shell of *Teredo* is not adequate to bore in wood, especially the shell of the larva, which is extremely fragile (*Hist.*, pp. 78–79). Rather is the boring accomplished (p. 86) by a kind of suction with the foot, aided by the action of the water which is taken into and forced out of the burrow.

Adanson (1759, pp. 268–269) remarks disparagingly on the views of Sellius, asserting that a slight movement of the shell, often repeated, is an entirely natural and sufficient explanation of boring. Such a movement he thought was occasioned by the rhythmic passage of the water in and out of the body through the siphons. Reimarus (1773, p. 55) properly attributed the movements of the shell to muscular action, and Osler (1826) noted the significant discrepancy in size between the anterior and posterior adductor muscles

Turton (1819) believed the secretion which hardens to form the calcareous lining of the tube of *Teredo* "to have a solvent action over the resin of the wood, and perhaps the fiber itself." Gray (1833), renouncing an opinion he had earlier set forth (1825) of a mechanical action on the part of the boring mollusks, affirms that the epidermis of the shell of *Teredo* is intact, whereas it would show abrasion if subject to continual rubbing against wood. The work of the rock borers, he avers, is accompanied by a dissolving of the calcareous rocks in which they bore; it is possible that the perforation of wood by *Teredo* is accomplished in some similar way.

Thompson (1835) set forth a theory which combines portions of the foregoing views. The foot, he states (p. 125), applying a solvent, "acts as a pioneer in mining a passage," which is afterwards enlarged by the action of the valves.

Hancock (1845 and 1848) attributed boring to the rasping of siliceous particles imbedded in the surface of the foot and mantle. He claimed to find such particles numerously in *Teredo norvegica,* in *Saxicava*, *Pholas,* and *Patella,* and published detailed descriptions and drawings of them. Unfortunately for this hypothesis, subsequent investigations have not confirmed the existence of such particles.

Home (1806, p. 281) appears to have first suggested that the foot of *Teredo* "adheres to the wood, acting as a center-bit while the animal is at work with the shell." This view of the function of the foot was adopted by Harting (1860), and has been accepted by a majority of writers since.

These represent the major hypotheses of the method of boring which have been advanced. Nearly every writer who has dealt with the Teredinidae has hazarded an opinion, but usually in substantial agreement with one of the foregoing. De Quatrefages (1849) assigned the function of boring to a fleshy fold of the mantle which overlaps the shell dorsally, aided perhaps by a chemical secretion, and possibly by siliceous particles such as Hancock had claimed to find. Deshayes (1850) favors the idea of a chemical solvent. Jeffreys (1862 and 1865), Dall (1895), Hedley (1901), and E. Kuhlmann (1914) consider the foot to be the boring tool, the function of the shell being to grip the sides of the burrow and so support the foot against the wood. Laurent (1850), Cailliaud (1856 and 1857), Petit-Thouars (1859), Baumhauer (1866), Grobben (1888), Lloyd (1897), Sigerfoos (1908), Redeke (1912, *a* and *b*), Moll (1914), W. Kuhlmann (1916), Calman (1919), Kofoid (1921), and Bartsch (1922), regard the shell as the effective boring instrument, the function of the foot being to hold the valves against the wood by attaching itself to the wall of the burrow.

THE BORING MECHANISM

The boring of *Teredo,* most writers agree, is accomplished by means of the shell and foot. The controversy of two hundred years has been not so much over the tools of boring as over the method of their use. Inasmuch as a study of the structure of these parts sheds considerable light upon their function, the evidence from structure will be treated first, and subsequently the evidence from observation and experiment.

THE SHELL

The boring lamellibranchs in general—*Pholas, Zirfaea, Xylophaga, Martesia*—exhibit a considerably modified shell, corresponding to their unusual mode of life. In *Teredo,* and the closely allied genus *Bankia,* this specialization of the shell has been carried to an extreme, so that, except in the veliger and early post-larval stages, its protective function is entirely lost. The shell of the adult *Teredo* has been reduced, relative to the size of the animal, until it covers only a small portion of the anterior part of the body, the much elongated visceral and branchial portion protruding posteriorly, and having as its protection the smooth, nacre-lined walls of the tapering burrow. Hedley (1901), emphasizing the supposed function of the foot in boring, regards this reduction of the shell as a degeneration, of which the next step would involve its complete disappearance. This assumption seems hardly justified in view of the elaborate modifications which characterize its structure.

The shell of *Teredo* is an irregularly shaped, subglobular structure, made up of two valves, united dorsally by a reduced ligament. The shell gapes widely in front (pl. 3, fig. 1) for the protrusion of the foot, and somewhat less widely behind for the backward extension of the long, slender body.

Each valve is made up of three rather distinct lobes, an *anterior,* a *median,* and a *posterior.* The last-named portion is frequently referred to as the *auricle,* a term which will be used throughout this paper in order to simplify so far as possible a confusing terminology arising from the fact that the median lobe of the shell must be further subdivided into three areas, an *anterior median*, a *middle median*, and a *posterior median*. The areas designated by these terms are indicated in figure A.

The margin of the anterior lobe meets that of the anterior median area at nearly a right angle. Each of these two areas is traversed by a number of prominent, denticulated ridges, parallel to its margin. The ridges of the anterior lobe accordingly meet those of the anterior median area at approximately right angles, forming a close-set series of such angles across the shell, and giving the impression of a line of suture, although the ridges are morphologically continuous.

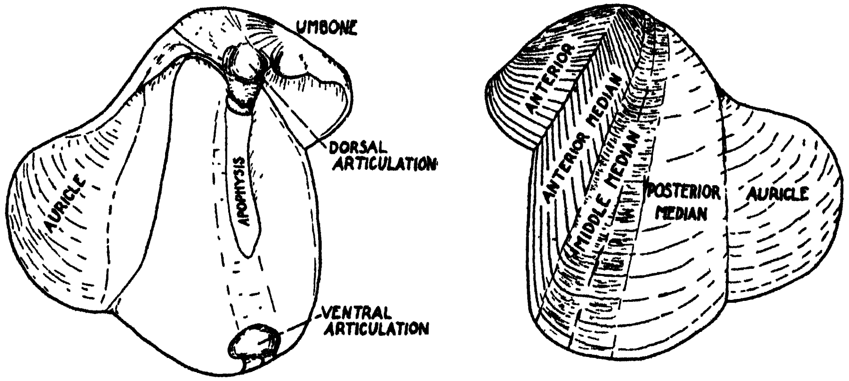

Fig. A. Interior and exterior views of the shell of *Teredo*, showing terminology used in description (from Miller, 1922).

The ridges of the anterior area are somewhat more widely spaced than those of the anterior median (pl. 3, fig. 2). The denticulations of the anterior lobe are merely fine serrations along the comb of each ridge. The denticulations of the anterior median area are rather intricately sculptured, wedge-shaped structures, the points of which are directed outward and backward. They are on the whole regularly spaced, and are much larger and coarser than the denticulations of the anterior lobe.

Ventrally and posteriorly, these ridges of the anterior median area become suddenly less prominent; the denticles disappear, and the ridges curve sharply and continue across the middle median and posterior median areas and the auricle as ordinary lines of growth. The middle median area represents the region of sudden curvature of the diminishing ridges, and is characterized by a more or less marked roughness. The posterior median area and the auricle are relatively smooth, and are separated by a rather well defined dorso-ventral depression.

The interior of the shell (fig. A) presents several features of interest. The recurved umbonal region, the dorsal knob, and, on the left valve, the ligament tooth or chondrophore beneath it, are readily homologized with similar structures in *Mya* and other lamellibranch types. The *apophysis*, a broad, flat process extending ventrally from beneath the chondrophore, apparently has, however, a homologue in only one other molluscan group, namely, the Pholadidae. The specialized knob by which the valves articulate ventrally is a structure found in the Teredinidae alone.

The division of the shell into three major lobes is apparent from within as well as from without. The forward edge of the auricle forms a prominent shelf by overlapping the posterior median portion.

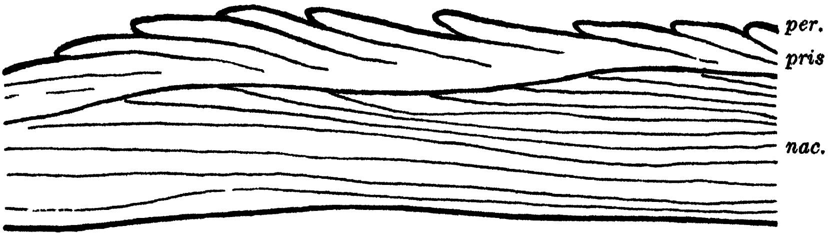

Fig. B. Cross-section through posterior median area of the shell of *Teredo navalis*. × 118. Abbreviations: *per.*, periostracum; *pris.*, prismatic layer; *nac.*, nacre.

A cross-section through the posterior median portion (fig. B) shows the shell of *Teredo* to have the typical laminated lamellibranch structure, with thin periostracum, prismatic layer of medium thickness, and an inner nacreous layer which in the older parts of the shell becomes very greatly thickened by addition of successive strata laid down by the portion of the mantle that lines the shell. The general structure differs in no substantial respect from that of the freshwater pearl mussel (*Margaritana margaritifera* Linn.) as figured by Rubbel (1911), or that of the Ceylon pearl oyster (*Margaritifera vulgaris* Schumacher) as figured by Herdman (1904) and Jameson (1912).

Of especial interest is a section through the anterior median area, cutting across the denticulated ridges. The gross structure of the shell presented in such section, as seen in figure C, exhibits a considerable modification of the structure above described. The periostracum is merely a thin line, scarcely visible in many cases; it is thicker between the denticles than over their surfaces. The prismatic layer occurs in a series of pockets, separated by partitions of nacre made up of a number of thin laminae. This traversing of the prismatic by the nacreous substance is a peculiar arrangement, and apparently a departure from the typical lamellibranch condition. Rubbel's (1911) figure of the shell of *Margaritana* in cross-section indicates strata of nacre projecting very slightly between the layers of prismatic substance, but giving no suggestion of rendering the prismatic layer discontinuous. In *Teredo* the secreting border of the mantle evidently remains extended well around the newly formed edge of the shell, reënforcing it with several layers of nacre before the following prismatic layer is deposited.

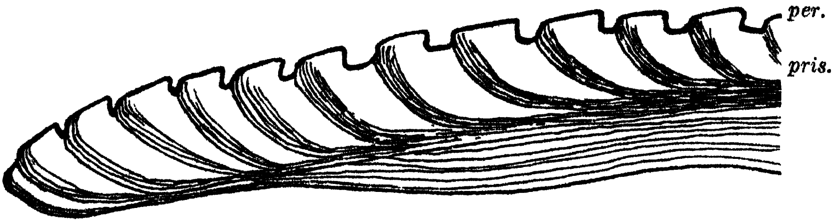

Fig. C. Section through the anterior median area of the shell of *Teredo navalis*, cutting across the denticulated ridges. × 118. Abbreviations as in figure B.

The attachment of the mantle to the shell is shown in plate 4, figure 6. The border of the mantle consists of two parts, an inner, free fold (seen as a translucent circular web in plate 3, figure 1), and an outer secreting fold, in a pocket of which the periostracum is secreted, and in which it is seen attached. In the formation of the shell the periostracum is evidently deposited first (Herdman, 1904, p. 40) and the prismatic layer subsequently, stratum by stratum.

There seems to be no doubt that the denticulated ridges, viewed in cross-section, are simply exaggerated growth ridges, and homologous with the growth ridges of such forms as *Mytilus* and *Mya*. These ridges owe their origin to alternating periods of growth and inactivity, correlated perhaps with variations in the plankton food supply, which appear to be rhythmic in nature (Kofoid, 1908), or with other conditions.

The rows of individual denticles present greater difficulty, but would seem to be homologous with the dorso-ventral striations characteristic of *Pecten, Cardium, Arca, Paphia,* and a variety of other forms. Indeed, an inspection of the wedge-shaped prominences on the shell of *Paphia,* produced by dorso-ventral striations cutting across the growth ridges, suggests immediate homologies with the rows of denticles on the shell of *Teredo*, a view which is strengthened by comparing both with an intermediate form, such as *Zirfaea* or *Pholas*.

Sigerfoos states (1908, p. 205), with regard to the deposition of these ridges on the shell of *Teredo*, "after growing a small amount, the anterior border has cemented to it a row of teeth which have been secreted separately in small pockets in the epithelium of the anterior edge of the mantle." The basis of this assumption is not stated, and there is nothing in the structure of the denticles to indicate that they have been thus secondarily attached to the shell. Rather do they appear to be an intrinsic part of it, produced by what is only a slight departure from the usual lamellibranch process of growth.

The two valves of the shell of *Teredo* are brought into contact with each other by the original dorsal and the secondary ventral knobs (fig. A). The anterior and posterior adductor muscles, opposing each other, enable the shells to rock back and forth upon these two points of contact, executing a movement about the dorso-ventral axis instead of the simple opening and closing movements of which alone most bivalves are capable. Thus a relatively slight modification of the shell, the development of a ventral knob or bearing, enables *Teredo*, without change of musculature, to execute an entirely new movement. The major axis remaining fixed, the valves of the shell are able to move back and forth with the alternate contractions of the anterior and posterior adductor muscles, somewhat like a pair of jaws moving laterally instead of vertically. The edges of the shell, of course, are never brought together, but gape widely in front to permit the protrusion of the foot even when the anterior adductor is tightly contracted. The movement of the valves is limited, the edge of each one being able to describe an arc of from twenty to thirty degrees, varying somewhat with the individual specimen.

With this understanding of the method of movement of the shell, the reason for the peculiar development of the auricle becomes apparent, its purpose being to serve as an attachment for the posterior adductor muscle, which is thus able to function more efficiently because of the increased leverage obtained by this backward extension of the shell. The margin of the auricle is usually slightly flexed outward, so that its edges do not cut sharply into the viscera when the backward margins of the valves approach each other on contraction of the posterior adductor.

The apophysis (fig. A) almost exactly parallels the dorso-ventral axis of motion of the shell, with which axis it is in the same plane from anterior to posterior. Thus its position is such that the movements of the valves affect it but very slightly. The use of the apophysis is to provide visceral support and to serve as a point of attachment for certain muscles of the foot, a function which would be disturbed if the apophysis underwent much movement with the rocking of the valves.

MUSCULATURE OF THE SHELL

The shell is provided with a large posterior adductor and a much reduced anterior adductor muscle. The latter was overlooked by most of the earlier investigators, including Deshayes (1848) and De Quatrefages (1849). Sigerfoos (1908) and Moll (1914) attribute its discovery to Grobben in 1888; but it appears that the credit should go to Osler, who in 1826 commented upon the notable discrepancy in size between the anterior and posterior adductors.

The posterior adductor is a large striated muscle, which has its attachment over nearly the entire inner surface of the auricle. Its structure is exceedingly firm and compact, fitting it to exert a powerful tension on the shell. In sagittal section (pl. 4, fig. 3) the area of the posterior adductor is nine times that of the anterior adductor. Near its attachment to the shell, the posterior adductor becomes expanded, so that in cross-section its area may be as much as thirteen times that of the anterior muscle. As the length of the posterior adductor in the contracted state is about three times that of the anterior adductor, its volume is accordingly at least thirty times that of the latter. The coarse, granular nature of the posterior adductor and, in the living animal, its distinctly reddish color, indicate that it is a muscle which accomplishes powerful contractions.

The great development of the posterior adductor of *Teredo* relative to the size of the shell, is seen from figure D, in which the areas of attachment of the shell muscles of *Teredo* are compared with those of *Mya*.

The relative development of the two muscles and the positions of their attachment with reference to the center of movement of the valves indicate beyond doubt that, whatever be the function of the shell in boring, the valves encounter opposition on the outward movement of their forward edges, but can be brought together again by the relatively slight pull which can be exerted by the small anterior adductor.

THE FOOT AND ITS MUSCULATURE

The foot of *Teredo,* in its contracted state, is a subcircular disc, the periphery of which is thrown into folds and is raised somewhat above the plane of the central area. A median notch in this wrinkled periphery, dorsal and ventral, marks the division between the muscle insertions of the right and left sides. The general appearance of the foot in a state of extreme contraction may be seen in plate 3, figure 1.

In sagittal section (pl. 4, figs. 3, 4, and 5), several differences are to be noted between the central disc of the foot and this peripheral area. The former is relatively smooth, non-ciliated, non-glandular; the latter is folded and wrinkled, strongly ciliated, and characterized by numerous close-set, deeply staining gland cells.

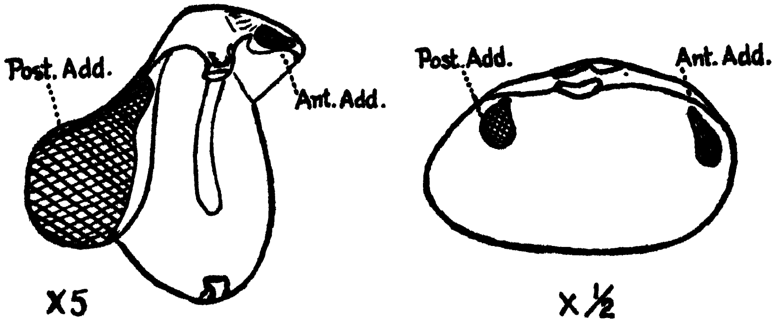

Fig. D. Relative areas of attachment of the anterior and posterior adductor muscles of *Teredo navalis* (left) compared with those of *Mya arenaria* (right).

The interior of the foot, with the exception of the muscular portion discussed below, is of an open, spongy texture, made up of loosely arranged connective tissue cells which permit an easy movement of body fluids among them for the purpose of modifying the turgor of the foot in process of extension or retraction.

The crystalline style of the digestive system lies just back of the foot, approaching it so closely as to be visible through the transparent tissues in a living animal.

The muscles of the foot are apparently entirely homologous with those of the lamellibranch type, with some modifications, as would be expected from the peculiar shape of this organ in *Teredo.* They consist of a pair of anterior retractors, a pair, or rather a series, of posterior retractors, and a pair of protractors.

The anterior retractors, extending dorsally from the foot, have their insertion in the anterior umbonal region of the shell. The posterior retractors, extending as wide, semicircular bands from the margins of the foot, are attached to the apophyses, which, extending ventrally from beneath the dorsal knobs of the shell, almost encircle the foot. The protractor muscles, which function in extending the foot by compressing the anterior portion of the body, have their insertions near those of the anterior retractors. Their relatively small size, as compared with the wide bands of retractors, suggests that the foot is extended as much by turgescence as by muscular action.

In specimens of *Teredo* removed from their burrows and kept in open dishes in the laboratory, the foot has sometimes been seen distended to a distance of two or three millimeters beyond the margins of the shell, and extremely turgid, so as to be almost transparent. Different areas of the foot appeared to be independently movable, so that the organ could be compressed on one side and expanded on the other, or rolled about somewhat amorphously and extended in any direction. All the movements observed were such as could be produced by the combined action of turgor and contraction of the semicircular bands of retractor muscles.

THE METHOD OF BORING

The foregoing morphological data have been set forth so far as possible entirely apart from any bias regarding the supposed functions of the organs described. Structure and function are, however, so closely correlated that a careful study of the former inevitably leads to certain conclusions concerning the latter. While anatomical evidence in such cases is necessarily inferential, it at least definitely limits the possibilities to be considered by showing that certain supposed functions can or cannot be performed.

The shell of *Teredo* has been shown to be a subglobular calcareous structure, gaping in front and behind, made up of two valves which are capable of rocking back and forth on specialized dorsal and ventral knobs. The anterior portions of these valves are equipped with sharply denticulated ridges, the projections of which are directed outward and backward. The musculature of the shell consists of a small anterior adductor and a very large posterior adductor, the two opposing each other in action.

The foot has been shown to be a subcircular disc, with a flattened sole and somewhat elevated, ciliated, glandular margin. Its musculature consists of protractors and anterior and posterior retractors, the last named being spread out in broad semicircular bands and attached to projections extending ventrally from the sub-umbonal region of the shell, and situated in the plane of least movement.

The structure of shell and of foot, when taken together, at once suggests two possible and almost equally plausible theories of their functioning in boring: (1) that the shell, by a spreading of the forward edges of the valves, acts as a clamp against the walls of the burrow to hold the animal in position, the boring being done by the foot; (2) that the foot acts as a sucker to hold the animal in place, the boring being done by the shell.

The former of these theories has been maintained by Jeffreys (1865) and Hedley (1901), and implied by Dall (1895) and E. Kuhlmann (1914), the line of argument being that the shells are inadequate to boring, that they do not show signs of wear, as would be expected if their function were that of rasping wood, that certain other forms, such as *Patella,* are known to bore with the foot, and that the walls of the burrow of *Teredo* are too smoothly polished to have been rasped mechanically; from which it follows that the only function possible to the shell must be to hold the animal in place while it is at work with the foot.

On careful examination, however, a serious fallacy is found in this hypothesis. The diameter of the anterior portion of the burrow is greater than the utmost lateral extension of the valves. This is rather apparent in consideration of the fact that the anterior portion of the tapering burrow is so much wider than the shell that *Teredo* when disturbed is able to retract fully a third of its length before the shell becomes jammed in the burrow.

The experiment was tried of opening longitudinally a burrow containing a living animal, which was left *in situ.* The shell was taken hold of with a pair of forceps and the auricles forced together as closely as possible, thus spreading the forward margins of the valves as would occur when the posterior adductor is contracted. It was found that the valves could not possibly be spread sufficiently to clamp themselves against the walls of the burrow (cf. pl. 5, fig. 7).

The greatest diameter of the shell has indeed been found by careful measurement to be actually less when the posterior adductor is contracted than when it is relaxed. This is because of the position of the axis of motion, which is anterior to center.

These considerations appear to demonstrate conclusively that, whatever be the function of the shell, it is not to support the animal in position by clamping itself against the walls of the tube. And without such anchorage by the shell, it is difficult to see how the foot could function at all in wearing away the wood.

The structure of the foot, furthermore, does not at all suggest a boring appliance. The ciliated periphery is made up of heavily staining gland cells. The sole of the foot is a single thin layer of columnar epithelium. If boring were accomplished by rubbing and wearing away the wood with the foot, the sole would be expected to be made up of thickened squamous or pavement epithelium.

The alternative hypothesis, that the foot acts as a sucking disc to hold the animal in position at the end of the burrow while it is at work with the shell, as pointed out in the review of literature, has gained a wider acceptance than any other. The elaborately denticulated shell at once suggests a rasping instrument, and the appearance of the foot (pl. 3, fig. 1) is certainly that of a sucking disc. The size and structure of the posterior adductor muscle and its attachment to a posterior elongation of the shell to obtain greater leverage, correlated with the development and arrangement of the denticulated ridges on the forward portions of the valves, point to the use of the shell as a boring implement. The modification of the foot is difficult to explain except as a specialization to serve as an organ of attachment.

Some of the objections that have been urged against this view, however, have considerable weight, and the question can hardly be settled except by direct evidence from observation and experiment. In the attempt to secure some definite proof as to the method of boring, investigations were pursued along the following lines:

EVIDENCE FROM MARKINGS ON THE WALL OF THE BURROW

The statement has been commonly made by advocates of the theory of boring by means of the foot that the smoothly polished burrows of *Teredo* show no evidence of having been rasped by the shell. Upon a close inspection of the extremity of the burrow under the microscope, however, it is common to find a series of fine scratches which give every indication of having been mechanically produced. These markings seem in general to have been overlooked by the earlier investigators. They were noted by Jeffreys (1865, p. 137), who denied the possibility of their being produced by the shell, without, however, suggesting any other explanation of their occurrence. Bartsch (1922, p. 4) has found such markings in palmetto wood only, and suggests that in the harder woods the scratches made by the projections of the shell may be lost by reason of the repeated toolings necessary in boring.

In the Douglas fir wood generally used for marine piling on the Pacific Coast, and in the pine blocks from the test boards planted under the auspices of the National Research Council Committee on Marine Piling Investigations, the writer has been able to find traces of the work of the shell in a considerable number of the burrows examined. These markings are usually somewhat obscure, and are easily overlooked amid the rugged corrugations presented under the microscope by the cut surface of the wood; they are best seen with the low power of the binocular microscope under a strong, carefully directed light.

In some instances such markings have been sufficiently distinct to be photographed, and this in the case of five different species representing the two principal genera of Teredinidae, *Teredo navalis* Linné, *Teredo diegensis* Bartsch, *Teredo affinis* Deshayes, *Bankia setacea* Tryon, and *Bankia mexicana* Bartsch (pls. 5 and 6). These markings are quite obviously mechanically produced, and cannot be explained except as scratches made by the rasping of the shell.

Jeffreys (1865) has stated that the striae at the tip of the burrow do not correspond to the ridges of the shell, and further that the shell cannot be brought into the position necessary for making such scratches, an objection which had previously been urged by Sellius (1733) and Hancock (1848).

A little experimentation with an animal *in situ* in an opened burrow will demonstrate that the cutting edges of the shell can be brought into contact with any portion of the cupped extremity of the burrow without difficulty, and without any violent bending or twisting of the body. But the question as to whether or not the scratches on the wood correspond to the ridges on the shell requires careful consideration.

In specimens where these markings are best preserved, they are seen to be of two kinds. The first (pl. 5, figs. 9 and 10; pl. 6, fig. 11) of these consists of fine scratches radiating in all directions from the latest center of boring; in some cases the center appears to have been shifted somewhat in the course of recent operations, so that the scratches produce an irregular criss-cross effect (pl. 6, fig. 13). Peripheral to these scratches occur somewhat coarser markings, parallel, close set, in series which overlap one another. Both sorts of markings are to be seen in plate 6, figure 14.

In order to determine whether or not such markings could be made by the ridges of the shell in the supposed normal movements of that structure, a number of shells were gently pressed into soft clay, moved a little in a manner intended to simulate a single stroke in boring, and then carefully withdrawn. In the clay impressions thus made, the marks left by the anterior portion of the shell were found to correspond in appearance and spacing to the scratches at the tip of the burrow, while the marks left by the denticles of the anterior median area of the shell corresponded to the coarser, although more close-set, peripheral striations in the burrow.

The arrangement of these two sorts of markings in the burrow indicates that the shell operates in boring in the following manner:

The anterior tip of the burrow is mined out by the anterior portion of the shell; the movement of the shell is necessarily in a direction longitudinal to the ridges of this area, so that their serrate edges act upon the wood as so many small saws. It will be recalled that the serrations on the ridges of the anterior area are extremely fine (pl. 3, fig. 2) as compared with the denticulations of the anterior median, and hence better fitted to act as the advance boring edges. Also the shape and position of the anterior lobe especially adapt it to working in the extreme tip of the cupped end of the burrow. The broader peripheral striations indicate that while the ridges of the anterior area are working saw-fashion in the tip of the burrow, the coarser wedge-shaped teeth of the anterior median area are at the same time working rasp-like, enlarging the diameter of the burrow and advancing the peripheral portion of its cupped extremity. Thus, while the tools might be compared to saw and rasp, their work is in effect that of drill and reamer.

The different series of striations and their often criss-cross effect signify that the position of the shell must be altered frequently in boring, and the valves applied to different portions of the extremity of the burrow. Indeed, in order to produce the markings shown in figures 9 and 10, plate 5, and figure 11, plate 6, the shell would have to be rotated by slow stages about the longitudinal axis through 180 degrees in each direction.

The peripheral corrugations attributed to the work of the anterior median area of the shell are rarely to be distinguished clearly, owing, no doubt, to a tendency of the coarse, close-set denticulations of this area to abrade and tear the macerated surface of the wood rather than to leave a clean-cut impression, as do the finely serrate ridges of the anterior portion of the shell. Also, the ridges of the anterior lobe cut longitudinally, whereas those of the anterior median merely scrape horizontally; thus the latter have less opportunity to leave a well-defined imprint on the wood.

EVIDENCE FROM EXAMINATION OF WOOD PARTICLES IN THE CAECUM

In support of the theory of mechanical boring by means of the shell, Harting (1860) pointed out that the shape of the wood particles taken from the caecum gives evidence of their having been cut off by the denticles of the shell, which are so disposed, he states, as to cut off minute quadrilateral chips. It is doubtful that the chips correspond as closely to the cutting edges of the shell as he maintained; but they do give evidence of having been mechanically produced. They are somewhat irregular in shape, some being quadrilateral, others elongate and fibrous, and still others finely granular. The elongate fibers appear sometimes to be cut in the direction of the tracheids of the wood, at other times to be cut across them, so that a cell wall or two may be detected in the fiber (Dore and Miller, 1922, pl. 18). This probably depends upon the direction of boring, whether the animal is going across the grain of the wood, or parallel to it.

Grains of sawdust would not be expected to correspond exactly to the interspaces between the teeth of the saw producing them; but a coarse saw will cut larger grains of sawdust than a fine saw. The occurrence of particles of different sizes in the caecum is significant. Large particles stand out conspicuously, from 0.30 to 0.40 mm. in length, and from 0.02 to 0.08 mm. across. On the other hand, minute particles, 0.02 mm. or less in length and of approximately the same breadth, occur numerously. While many particles of intermediate size indeed occur, one is able to differentiate readily the two classes of particles, elongate, fibrous ones and fine granular ones. The conclusion immediately suggests itself that the latter are produced by the fine serrations of the anterior lobe of the shell, while the former are rasped off by the coarser denticulations of the anterior median area.

EVIDENCE THAT THE SHELL IS CAPABLE OF BORING

Whether or not the shell of *Teredo* is capable of accomplishing the work of boring has been a subject of much debate. It was shown by Cailliaud (1856, p. 21) by a practical experiment that wood can be successfully rasped away by scraping under water with the shell, and Jeffreys (1865, p. 135), although he believes the foot to be the instrument of boring, admits that he repeated this experiment of Cailliaud with success.

It has been maintained by Gray (1833), De Quatrefages (1849), Deshayes (1850), Jeffreys (1865), and others, that the delicately sculptured shell of *Teredo* is characteristically well preserved, with the denticles and even the periostracum intact, whereas if it were used for boring purposes the surface would most certainly show signs of wear. Harting (1860) undertook to explain away this objection by showing that the more recent rows of denticles only are used in boring, the older rows being out of use and hence not subject to further wear. He takes issue, however, with Deshayes and De Quatrefages in regard to the supposed perfect preservation of the shell, asserting that the surface does show considerable indication of wear.

In this Harting was in a measure correct. Shells of *Teredo* frequently exhibit broken denticles, sometimes over a considerable area; and it is by no means uncommon to find a shell that has been fractured and subsequently repaired, leaving an irregular scar. As the shell is protected from peradventure of such accidents from outside causes, there seems to be no other explanation than that they result from the animal's encountering areas of more than ordinarily hard wood, and exerting greater muscular power in the effort of boring than the brittle shells are able to bear. The shells and their denticles, while extremely hard, are very brittle, and are more easily broken than worn away, as one discovers on attempting to grind thin sections of them.

By no means all shells of *Teredo* exhibit this impaired condition, however, the majority being found in a good state of preservation, with regular, perfectly sculptured denticles, as opponents of the theory of boring by the shell have pointed out. The argument based on this fact has, on first reflection, considerable weight.

That boring with the shell of necessity involves a notable wearing away of the tool is not, however, to be accepted as an axiom, and it does not seem to be supported by proof. The writer has tried the experiment of rasping the surface of a piece of Douglas fir wood under water with a medium sized teredo shell held between the thumb and forefinger. By this method a depression 6 mm. in diameter and 1.2 mm. deep was made in thirty minutes, at the end of which time the denticles of the anterior and anterior median areas, examined under the microscope, showed not the least trace of wear.

It is to be assumed that *Teredo* is able to apply its shell with rather more efficiency than were the fingers of the experimenter. Furthermore, the brunt of boring is not carried by one set of denticles for more than a rather brief period of time. It has been shown (Miller, 1922, p. 300) that a new cutting edge is regularly added to the shell of *Teredo navalis* from two to four times a month, and during the early stages of boring much more frequently. As with the addition of new ridges to the margin of the shell the older ones pass out of use (see pl. 4, fig. 7), there seems no reason to suppose that, boring in ordinary pine or fir wood, the denticles would of necessity be visibly worn down by their work. The cases in which worn or broken denticles occur are exceptions to the general rule.

Possibility of the Use of a Chemical Solvent in Boring

The possibility of the use of a chemical solvent as an aid in the boring of *Teredo* has been invoked many times. By the earlier writers, in general, it was not favorably regarded. Sellius (1733) thought that a 'corrosive liquor' would be more likely to destroy the shell of the animal than to dissolve the wood. Reimarus (1773) objected to the solvent theory on the ground that the burrows of *Teredo* approach one another so closely and yet the thin intervening partition is not broken through; the action of a solvent, he thought, could not be so carefully controlled. The nineteenth century, however, found a number of exponents of the theory of boring by chemical means—Turton (1819), Gray (1833), Thompson (1835), De Quatrefages (1849), Deshayes (1850), and others. But attempts to demonstrate the presence of an acid or other secretion in *Teredo* which might serve the purpose in question were unavailing, and the assumption of a chemical action on the wood rests rather on speculation than on observation or experiment.

The composition of wood approximates 50 per cent of cellulose and 25 per cent of lignin, the remaining 25 per cent being made up of xylan and small quantities of hemicelluloses, soluble sugars, tannins, and resins. The most feasible method of 'softening' wood is by a reduction of the cellulose, which gives to wood its toughness. Lignin, the other principal component of the cell walls, is probably impervious to attack by any substances organically produced, being insoluble in 72 per cent sulphuric acid (König, 1913) or in 5 per cent sodium hydroxide solution (Dore, 1920). Xylan is soluble in both these substances, but probably not in any concentration possible in a living organism. Even if the xylan were removed, its absence would hardly facilitate boring. The same may be said regarding removal of sugars, tannins, and resins. In the organic decomposition of wood, hydrolysis of cellulose is the usual method in nature. Decay of wood proceeds by the reduction of cellulose by fungus action, the lignin content usually, if not always, remaining intact.

The fact that *Teredo* is known to produce enzymes capable of hydrolyzing cellulose (Harrington, 1920; Dore and Miller, 1923) suggested the possibility of some such enzymic action in the immediate region of boring. The glandular nature of the foot suggested that structure as an agent for secreting a cellulose-dissolving enzyme and applying it at the point of action.

It would seem that the action of such an enzyme, if it occurs, should spread at least for a limited distance through the cells of the wood immediately adjacent to the extremity of the burrow. The tracheids of Douglas fir are from one to three millimeters in length, and it is hardly conceivable that a cellulose-dissolving enzyme applied at one end of a tracheid should not spread through its entire length. Probing in the extremity of the burrow with a needle, however, did not reveal any area of softened wood, as would be expected on this hypothesis. The walls and end of the burrow were quite as hard as any unattacked portion of the wood.

Hematoxylin being a selective stain for cellulose (Molisch, 1921), thin shavings were cut from the wood at the end of the burrow, and this stain applied by micro-chemical methods. It was expected that any diminution in the cellulose content of the wood at the point of latest boring would be recorded by a lighter staining of the cells in this region, as qualitative differences in staining with this reagent apparently afford an index to relative amounts of cellulose present (Lazier, 1924). The most careful staining, however, revealed no differences between the wood immediately adjacent to the burrows and that farther away.

It was further attempted to compare quantitatively the composition of shavings from wood immediately adjacent to burrows of *Teredo* with that of shavings from sound portions of the same timber. Samples for this purpose were carefully prepared from a section of Douglas fir piling of which the outer four or five inches were entirely riddled by *Teredo navalis,* but of which the heart was still sound. The peripheral sap wood was not included in the sample taken from the bored area; this sample was limited to fragments from the ends of the burrows or from portions of the wood which were so honeycombed as to leave only walls hardly thicker than paper between adjacent burrows.

An analysis of these samples was very kindly made by Professor W. H. Dore, of the Laboratory of Plant Nutrition of the University of California. The following figures represent the averages of duplicate analyses in each case:

	Results in percentages	
	Sample of wood from wall of burrow	Sample of sound portion of wood
Moisture	3.95	3.93
Benzene-alcohol extract	1.81	3.77
Cellulose	53.37	53.29
Lignin	33.53	31.98
Undetermined	7.34	7.03

The difference in percentage composition of the benzene-alcohol extracts of the two samples is attributed to the fact that the sugars, tannins, and coloring substances which, with resin, compose this extract, are largely water soluble, and hence would naturally be leached out of the honeycombed portion of the wood more quickly than from the sound portion.

The significant figures here are those for cellulose and lignin, which are nearly identical in the two cases, and accord well with the figures given by Dore and Miller (1923) for the normal composition of Douglas fir wood after being subjected to the leaching action of water.

While the writer does not consider that the possibility of the use of a glandular secretion to facilitate boring is definitely disproved by these experiments, the evidence does not favor the assumption of such an agent. The idea of a chemical action on the wood is not necessarily a corollary of the theory of boring by the shell, since the effect of water alone is sufficient to greatly soften wood. An attempt to rasp dry wood with a shell of *Teredo* resulted in the speedy destruction of the shell, whereas under water such rasping was readily accomplished, as above described. There seems to be no reason to suppose that, with the wall of the burrow continually softened and macerated by the presence of water, *Teredo* should have any difficulty in rasping away the wood by the action of the shell.

OBSERVATIONS ON ANIMALS IN OPENED BURROWS

Notwithstanding the fact that *Teredo* is one of the most hardy of marine organisms, adapting itself readily to a wide variety of conditions, it is very easily discouraged from boring by any disturbance to its burrow. Animals removed from the wood will apparently under no circumstances bore again. Sellius (1733) and later Laurent (1850) tried the experiment of removing animals from their burrows and placing them in wooden tubes; but no boring was done under these conditions.

The writer has a number of times opened burrows near the anterior end and kept the borers under observation in the hope that some movement of the shell might be observed which would shed light on the method of boring. But teredos thus exposed would first contract and remain quiescent for several days, then, if the injury to the burrow were not too great, repair the breach with a wall of nacre and subsequently bore in another direction. There was never any indication of boring so long as an opening remained in the wall of the burrow.

The experiment then suggested itself of opening the burrow and sealing it over with a piece of glass, with the thought that the teredo, thus protected, might continue boring without first closing itself from view by an opaque wall. The procedure adopted was to open the burrow carefully at the anterior end by taking off thin slivers of the wood until an aperture of the desired size had been made. Almost at the first disturbance of the burrow, the occupant would retract about one-third of its length, so that the anterior part of the burrow could be tooled as desired without danger of injury to the animal. The edges of the opening were planed down and a glass cover-slip was applied and sealed around the margins with vaseline. The block of wood containing the specimen was then placed in a clear glass museum jar filled with salt water.

Of seven specimens prepared in this way, only one afforded any data. This one, the burrow of which had been opened up longitudinally for a distance of rather more than an inch from its anterior termination, after three days of inactivity in the contracted state began slowly advancing along the burrow. It became extended about a half-inch during the day, then retracted again. The following day it became more active, advanced along the burrow and turned slowly about from side to side.

A binocular microscope was adjusted in such a way as to give a good view of the animal's activities, and a strong concentrated light was thrown into the burrow with a concave mirror. At first it was feared that the light might be a disturbing factor, and observations were limited to a few seconds at a time. But as the organism appeared to be insensible to light, however concentrated, later observations were made continuously over considerable periods of time.

The animal appeared to advance along the burrow at times by feeble peristalsis, but more generally by crawling with the foot. This organ was observed to be surprisingly facile, capable of being much extended and put to a variety of uses. The teredo turned from left to right and back again a number of times, as though exploring different portions of the wall of the burrow with the foot. It was observed to turn through an arc of 90 degrees in less than one minute; then, after resting five minutes, it continued turning in the same direction through another arc of 90 degrees in about fifteen seconds; after another five minute period of quiescence, it continued turning, still in the same direction, through an arc of 60 degrees in about one minute. Thus its movements involved a total turning through 240 degrees in twelve minutes. After some minutes' quiescence, it turned about 20 degrees farther in the same direction, making a total of 260 degrees. In this process of turning, the mantle and viscera were very obviously twisted, apparently without any inconvenience to the animal.

A reverse turning was now begun. The shell was turned from left to right through about 160 degrees in one minute. This was followed by a period of rest of about seven minutes, after which another turning from left to right, of about the same degree, was executed in one minute. Ten minutes later the animal moved another 160 degrees in three minutes. Thus the reverse turn involved a total of 480 degrees, and occupied twenty-two minutes. In this position the mantle and viscera were visibly twisted in the opposite direction from that noted above.

These observations demonstrate that *Teredo* is readily able to turn through considerable distances about its longitudinal axis, as postulated previously (p. 56) from an examination of the markings on the end of the burrow.

In executing such turning movements, the foot several times passed directly across the glass, ventral side to the observer, so that the manner in which it functioned could be well studied. The organ was first flattened against the surface of the glass, and its margins spread out so that they extended beyond the edges of the shell. Then, apparently by contraction of the retractor muscles, the margins were rather suddenly drawn in, and the central disc of the foot slightly cupped, very obviously constituting a sucker. After each contraction, the foot sought a new attachment, moving laterally about 0.5 mm., and hitching the shell along to a new position upon the following contraction. Thus the turning of the shell was really accomplished by a crawling around the burrow with the foot. Each contraction of the foot occupied about one second, with an interval of one or two seconds between contractions for relaxing the foot and taking a new hold.

Extremely interesting to observe was the use made of the dorsal fold of the mantle which overlaps the top of the shell. The function of this structure has been long in doubt. De Quatrefages (1849) somewhat inappropriately termed it the "cephalic hood" (*capuchon cephalique*), and considered it to be the boring device, although its structure, consisting of loosely arranged connective tissue cells (pl. 4, fig. 3), is not at all adapted to such a use.

During the movements of the animal in the burrow as above described, this fold of the mantle was observed to be greatly distended by turgor, so that it filled up entirely the diameter of the burrow, and acted as a counter pressure to the foot. Thus this troublesome structure is found to have an important use. It doubtless further subserves the function suggested by Kofoid (1921, p. 27) of preventing the rasped-off particles of wood from getting between the mantle and the wall of the burrow.

After exerting itself rather aimlessly in the manner described at intervals throughout the day, at 11 P.M. the animal was observed to have advanced almost to the extremity of the burrow and to be executing what appeared to be boring movements with the shell. The valves were directed ventrally, and were moving rhythmically, at the rate of from eight to twelve times a minute. This was discontinued in about a half-hour, and the animal retracted slowly about one inch from the end of the burrow, there remaining inactive.

At 9 o'clock the following morning the shell was again observed to be in action. The animal was still retracted some distance from the end of the burrow, and the valves were directed ventrally, the body being bent at a right angle at its junction with the shell. The dorsal fold of the mantle was much distended, pushing against the roof of the burrow, and extending forward fully 2 mm. beyond the shell. The boring movements were not very persistent during the day, and no appreciable depression in the wall of the burrow had been made by evening. There were no further developments the next day; but on the morning of the following day the animal was found to have bored a considerable side passage, so that its shell was nearly out of sight. A quantity of bored wood had been discharged overnight by the siphons. By this time the teredo had begun to line the glass slide with nacre, and this prevented further observations. The method of boring, however, had been made clear.

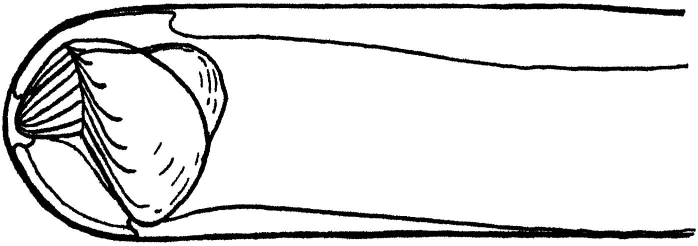

Fig. E. Boring position of *Teredo* in end of burrow.

The boring is accomplished by rasping with the valves, which are held in position by the combined action of the foot, attached to one wall of the burrow, and the dorsal fold of the mantle, pushing against the opposite wall. The typical boring position is seen in figure E (cf. also pl. 5, figs. 7 and 8). At each stroke of the valves the foot takes a new hold. As the backward stroke of the valves is completed, the foot is relaxed and its margins are spread out so far that they overlap the edges of the shell. Then, by a sudden contraction, the margins of this structure are drawn in and the valves brought forward into position for a new stroke. Then follows the slow, labored contraction of the large posterior adductor, causing the forward edges of the valves to spread apart and rasp the wood. That the valves are actively scraping the wood on this stroke is indicated by the fact that they have been observed frequently to slip, the backward margins being drawn together with a jerk instead of the usual slow, steady pull.

The alternate expansion and contraction of the foot during boring doubtless explains why the marks of the work of the shell in the anterior portion of the burrow are so often obliterated.

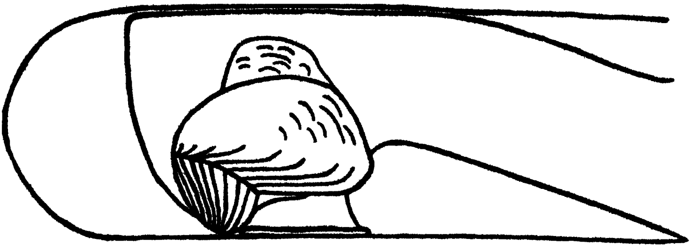

Fig. F. Boring position of *Teredo* beginning a side passage at a right angle to the former course of the burrow.

The position assumed by *Teredo* in boring off at a right angle to its former direction of progress is seen in figure F. One would hardly have supposed that such an awkward position is assumed by the animal in changing the course of its burrow, were this not actually a matter of observation. Apparently the valves function without difficulty under such circumstances. Abrupt changes in the direction of the burrow may often be found in heavily riddled piling.

The disposition of the rasped-off particles of wood it was not possible to observe, because of their minute size, and also because the animal did most of its boring on the side of the burrow away from the observer. There is, however, every reason to believe, as stated by Lazier (1924), that it is the function of the cilia of the periphery of the foot to sweep these particles up and into the range of the cilia of the oesophagus.

DISCUSSION

The foregoing observations are believed by the writer to establish the method of boring of *Teredo* beyond reasonable doubt, although the conclusions reached are contrary to the views he previously had held. The use of shell and foot as above described represents a complete departure from the normal usage of these structures in the lamellibranch group.

The burrowing habit is widely distributed among pelecypod mollusks—*Mya, Solen, Cardium, Platyodon, Unio,* to mention only a few typical examples. In all of these forms the burrowing is done by means of the foot, the shell serving only a protective function. In *Teredo* the condition is practically reversed, the shell being the instrument of boring and the foot an organ of attachment, ample protection being afforded by the medium in which the burrow is excavated.

The structural modifications involved in this alteration of the functions of shell and foot afford an extremely interesting study. It has been stated by Hedley (1901, p. 244) that the reduction of the shell of *Teredo* represents a degenerative change, the next step in which would be the total disappearance of the structure. Such an idea becomes untenable in view both of the high degree of specialization of the shell and of its importance in the economy of the animal. Degeneration connotes a change in the direction of less complexity. A structure so admirably adapted to serve a particular function as is the shell of *Teredo* cannot properly be regarded as in any sense degenerate.

What is perhaps the most interesting adaptive specialization presented by the teredinid shell is the arrangement by which the two adductor muscles are enabled to oppose each other in action. The prominent ventral knob which facilitates the rocking of the valves of the shell is apparently a character peculiar to this group, although on the valves of *Zirfaea crispata* and some other forms a slight ventral excrescence is developed for a similar purpose. In the myacid genus *Platyodon*, the shell gapes posteriorly to such an extent that a considerable rocking of the valves is possible through the opposed action of anterior and posterior adductors, and such movements have been noted in specimens under observation in aquaria. The valves rock ventrally merely on their edges, however, not having any specialized bearing developed. Even in a form as generalized as *Mya*, the valves of the shell are able to rock back and forth in this manner to a limited extent.

The foot of *Teredo*, as well as the shell, has been regarded as a degenerate structure: Dall (1895, p. 560) and Pelseneer (1906, p. 216), and others have expressed this idea. Neither the structure nor function of the organ, however, lends support to such a view. While one of the three pairs of anterior shell muscles with which primitive types such as *Yoldia* (Drew, 1899) are equipped appears to have been lost, the posterior retractors are developed in a more than compensatory degree, being spread out into broad, semicircular bands which have their insertion on a special process of the shell.

The structure of the foot as a whole is more complex than that of the usual lamellibranch type, and observations of the animal in its burrow indicate that the organ serves a variety of uses. It functions as a means of limited locomotion about the narrow confines of the burrow. Apparently it acts as an organ of sense, exploring the walls of the cavity, so that the animal in some way becomes aware of the proximity of other burrows and turns aside to avoid breaking through into them. It serves as an organ of attachment to give a purchase for the work of the shell, and occasionally to turn the shell to a new boring position. Its ciliated margins, which between every stroke in boring are spread out beyond the edges of the shell, sweep the rasped-off particles of wood up to the mouth.

As there are no retractor muscles attached to the central disc of the foot, the suction of this organ is apparently accomplished by alternate increase and decrease of turgor, for which the area of loosely arranged connective tissue cells back of the sole of the foot is obviously adapted. The suction exerted by the foot to hold the shell in place need not be very powerful, for two reasons: First, the effective stroke of the valves is the outward and backward one; the teeth on the ridges being directed backward, the shell in executing the boring movement tends to force itself forward, reducing very markedly the suction that would be necessary if the cutting were done on the forward stroke of the valves. Second, the suction exerted by the foot is not a direct suction from an attachment at the tip of the burrow; in the boring position the attachment of the foot is decidedly ventral, so that it has the advantage of friction as well as suction to hold the shell in position against the wood. The expanded dorsal fold of the mantle, acting as a counter pressure, further assists this office of the foot.

The Teredinidae as a group represent an extreme degree of adaptive modification to a peculiar mode of life. As regards shell, foot, and musculature, they present continually the most obvious homologies with what has come to be regarded as the lamellibranch type, while at the same time exhibiting unique specialization in all of these structures, corresponding to altered functions. As regards method of boring, they exhibit a rather typical lamellibranch habit carried on in an unusual medium and performed in an entirely new way. Throughout their entire organization, the members of this group present a remarkable coördination of structure and function adapted to a unique environmental complex.

SUMMARY

1. The method of boring of *Teredo* having long been under debate, the present investigation was undertaken with a view to securing definite evidence from observation and experiment.

2. A study of the shell and its musculature indicates that the two valves, rocking upon specialized dorsal and ventral fulcra which permit the anterior and posterior adductors to oppose each other, are equipped to perform a rasping action on wood.

3. The great development of the posterior adductor muscle, and the direction of the denticles on the shell, indicate that the outward stroke of the forward edges of the valves is the effective one in boring.

4. The specialized subcircular foot is not adapted to boring, the central disc being made up of a single layer of columnar epithelium, not squamous epithelium as would be expected in a structure subjected to extensive rubbing against the wood. The foot is, however, adapted to act as a sucking disc by a combination of turgor and the action of semicircular bands of retractor muscles.

5. Many burrows exhibit at the anterior end markings evidently of mechanical origin. These are of two sorts, fine, close-set scratches radiating from the center of boring, and coarser peripheral striations, corresponding respectively to the finely denticulated ridges of the anterior lobe of the shell, and the coarser denticulations of the anterior median area.

6. The nature of the markings indicates that the anterior lobe of the shell acts as the advance boring tool, the more coarsely denticulated anterior median area working peripherally to enlarge the burrow.

7. Particles of bored wood in the caecum afford evidence of having been cut off by the denticles of the shell.

8. The experiment of rasping wood under water with a shell held between the fingers demonstrated that a depression 1.2 mm. deep could be rasped in thirty minutes without the shell's showing any trace of wear.

9. The possible use of a chemical solvent to facilitate boring was investigated, with negative results.

10. Observation of an animal in a burrow which had been carefully opened and sealed over with a cover glass finally demonstrated that the boring of *Teredo* is accomplished by the shell, which is held in position by the combined action of the foot, attaching itself to the wall of the burrow by suction, and the dorsal pallial fold, distended by turgor, acting as a counter pressure against the opposite wall of the burrow.

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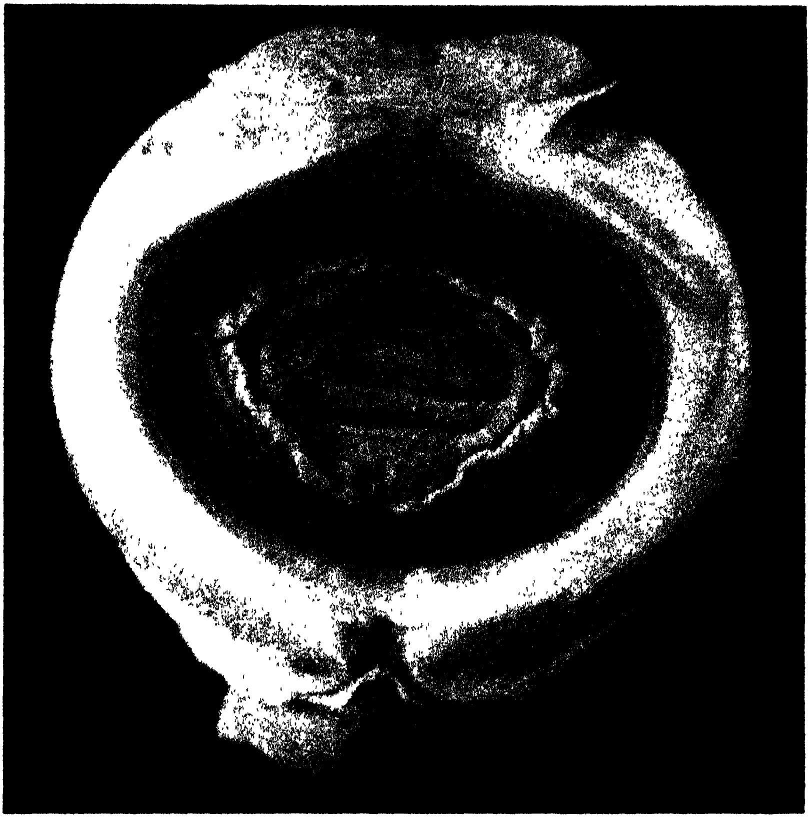
EXPLANATION OF PLATES

PLATE 3

Fig. 1. Anterior view of *Teredo navalis*, showing relations of shell and foot. The translucent marginal web of tissue encircling the aperture of the shell is the inner free fold of the mantle. × 10.

Fig. 2. Detail of surface sculpture of the shell of *Teredo navalis*, in the region of the angle formed by the juncture of the finely serrate ridges of the anterior lobe (left) with the coarsely denticulate ridges of the anterior median area (right). × 50.



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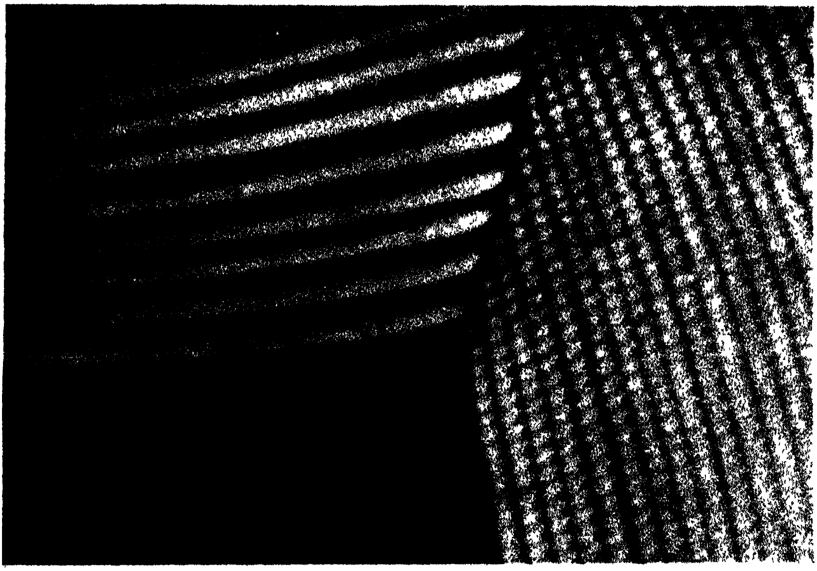


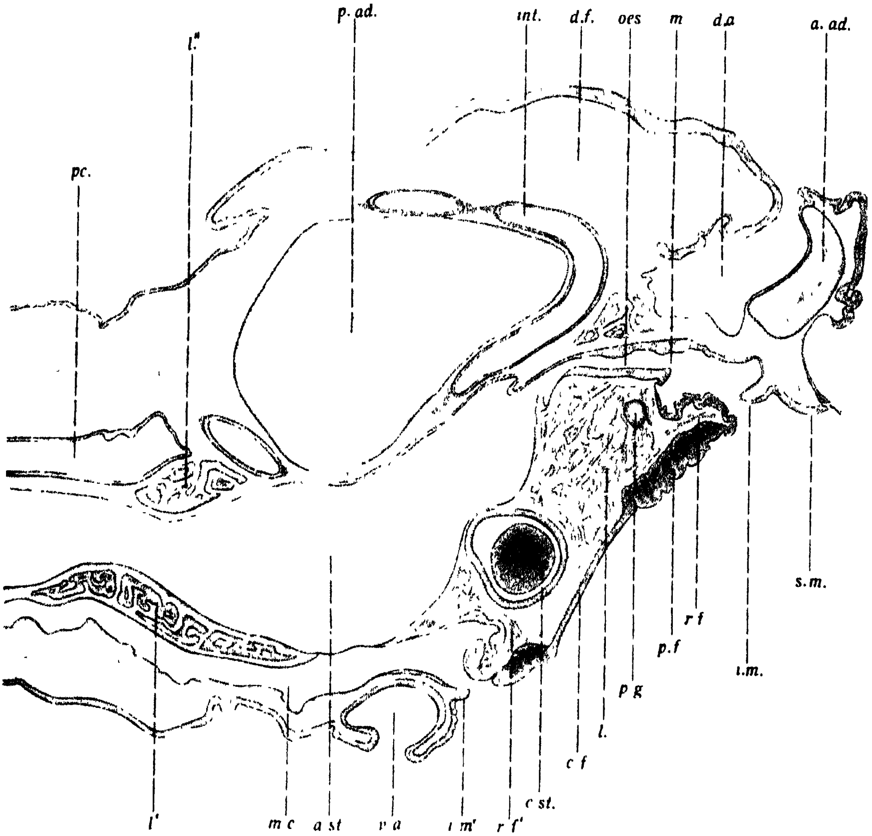
PLATE 4

Fig. 3. Anterior end of *Teredo navalis* in sagittal section. Camera, × 25. *a. ad.*, anterior adductor muscle; *a. st.*, anterior part of stomach; *c.f.*, central disc of the foot; *c.st.*, crystalline style; *d.a.*, pocket of the dorsal articulation of the shell; *d. f.*, dorsal fold of the mantle; *i. m.*, *i.m.'*, inner free fold of the mantle; *int.*, intestine; *l.*, *l.'*, *l.''*, livers; *m.*, mouth; *m. c.*, mantle cavity; *oes.*, oesophagus; *p. ad.*, posterior adductor muscle; *p. c.*, pericardial cavity; *p. f.*, peripheral area of the foot; *p. g.*, pedal ganglion; *r. f.*, *r. f.'*, retractor muscles of the foot; *s. m.*, secreting border of the mantle; *v. a.*, pocket of ventral articulation of the shell.

Fig. 4. Detail of epithelium of the central disc of the foot. Camera, × 750.

Fig. 5. Detail of epithelium of the periphery of the foot. Camera, × 750.

Fig. 6. Detail of cross-section through anterior termination of mantle (*i. m.*, *s. m.*, fig. 3). Inner, free fold (left), and outer secreting fold (right), showing insertion of periostracum. Camera, × 175.



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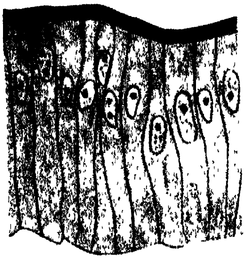

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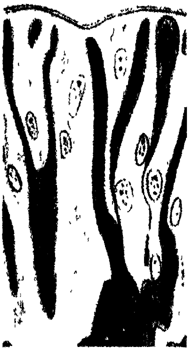

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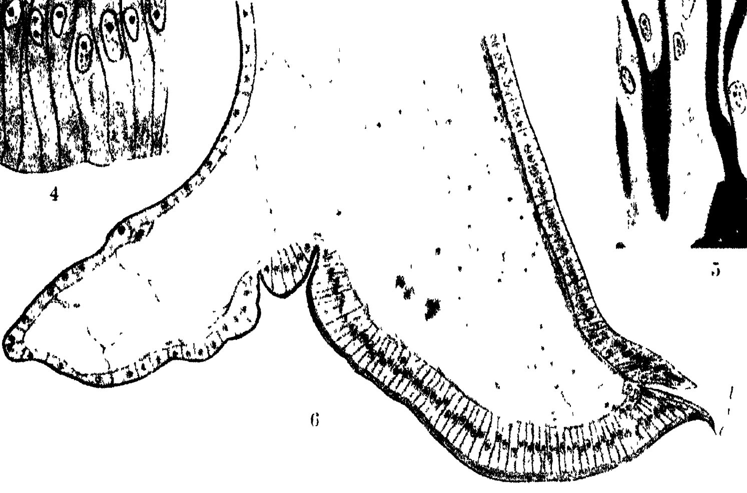

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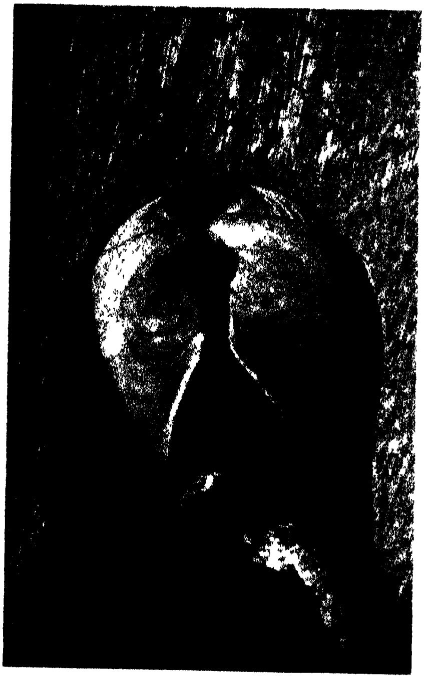
PLATE 5

Fig. 7. Shell of *Teredo navalis* mounted in position at end of burrow, dorsal view. × 10.

Fig. 8. Shell of *Teredo navalis* mounted in position at end of burrow, lateral view. × 10.

Fig. 9. Cupped extremity of a burrow of *Teredo navalis*, showing markings made by the shell. × 10.

Fig. 10. Same, smaller specimen.



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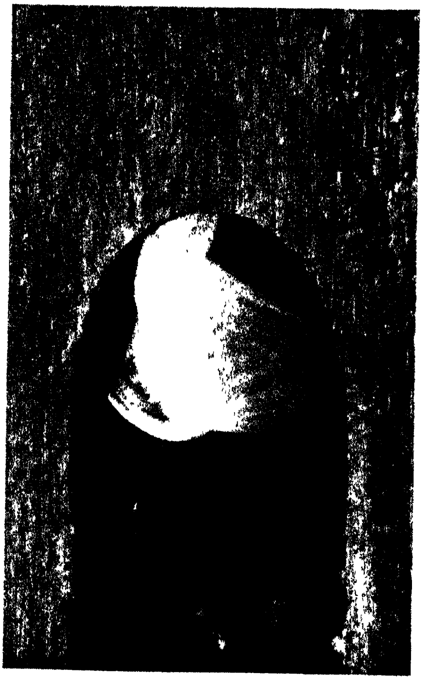

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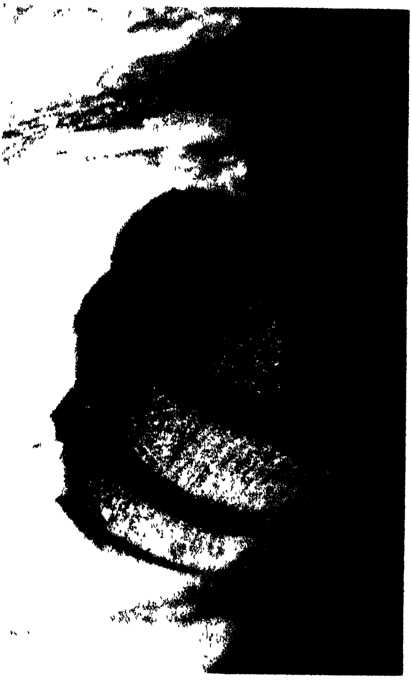

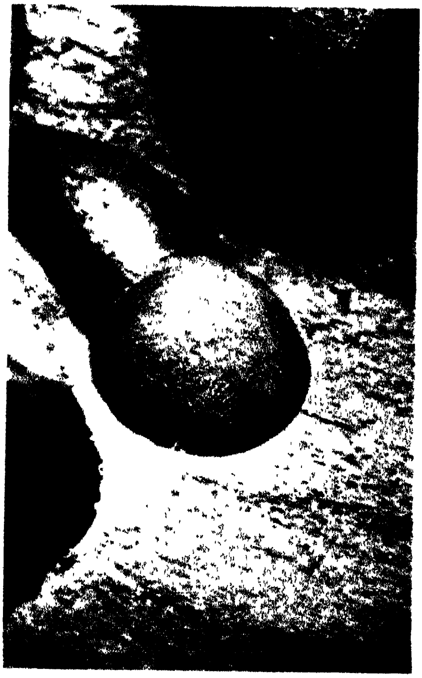

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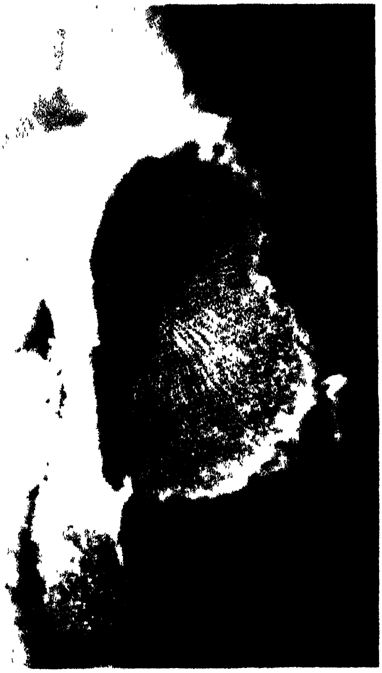
PLATE 6

Fig. 11. Cupped extremity of a burrow of *Teredo diegensis*, showing markings made by the shell. × 10.

Fig. 12. Same, *Teredo affinis*.

Fig. 13. Same, *Bankia setacea*.

Fig. 14. Same, *Bankia mexicana*.



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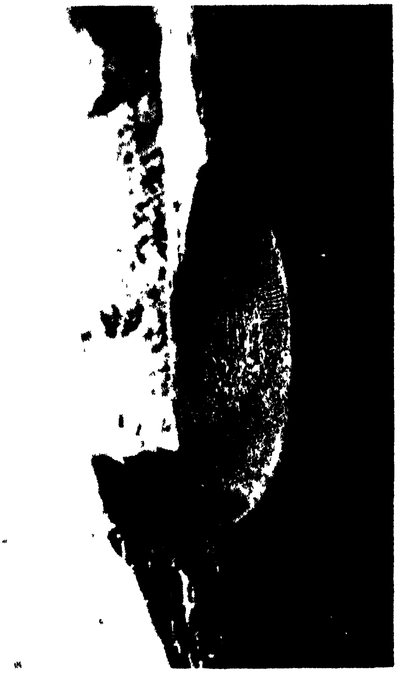

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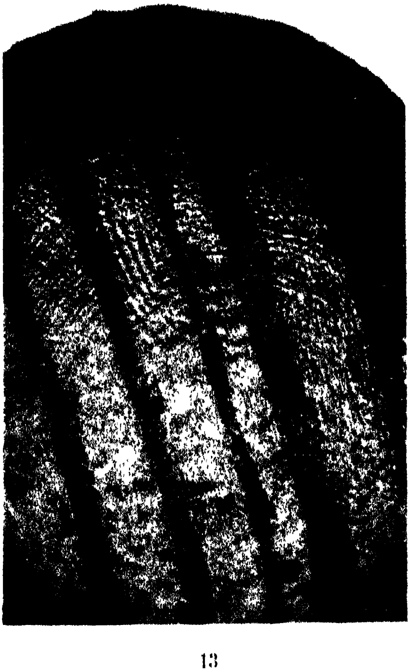

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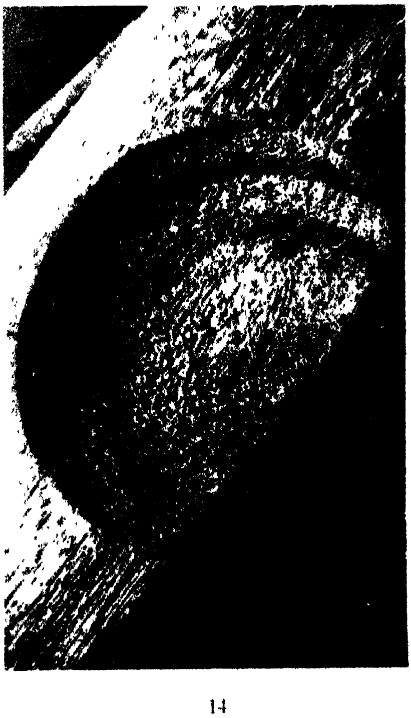

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THE FREE-SWIMMING COPEPODA OF SAN FRANCISCO BAY

BY

CALVIN O. ESTERLY

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FOREWORD

This paper contains descriptions and data on distribution of the Copepoda collected by the steamer "Albatross" of the United States Bureau of Fisheries during the years 1912 and 1913. These collections were made in the course of a biological and hydrographic survey of San Francisco Bay carried on conjointly by the United States Bureau of Fisheries and the University of California under the direction of Dr. Francis B. Sumner, naturalist of the Bureau of Fisheries, and Professor Charles A. Kofoid, of the University of California. The data pertaining to the physical conditions of the bay, such as temperature, salinity, and depths, have been published in the report of Sumner *et al.* (1914).

This report is the first account of the Copepoda of harbor and estuarine waters on the western shores of the American continents. No new species have been found in the collections, a condition anticipated in view of the cosmopolitan distribution of many of the Copepoda.

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PART I. DESCRIPTIONS OF GENERA AND SPECIES

The written accounts do not go into great detail, but it is hoped that they are clear and diagnostic. Only the more characteristic appendages are illustrated. There are drawings of parts that are not described in writing in cases where drawings are apt to be more useful than text. I have as a rule followed the plan adopted in the Siboga (Scott, 1909) and Ingolf (With, 1915) reports of not showing the 'feathering' on the bristles of the swimming feet and other appendages, since it is not of particular value in recognizing species.

In citing literature no attempt has been made to refer to any work previous to the appearance of Giesbrecht's great monograph (1892). The citations that are more recent than 1892 are of papers containing useful notes on distribution, structure, or relationships, or of papers giving extended descriptions.

The copepods were sent to me after they had been separated from the rest of the material collected.

Family CALANIDAE

Scott (1909), as a result of his study of the Siboga collections, lists the following genera under this family: *Calanus* Leach; *Canthocalanus* nov.; *Calanoides* Brady; *Megacalanus* Wolfenden; *Bradycalanus* nov.; *Undinula* nov. With (1915) in dealing with the Ingolf collections has put the following genera in the Calanidae: *Calanus* Leach; *Macrocalanus* Sars; *Megacalanus* Wolfenden.

It is apparent from the discussion given by Scott and by With that there is considerable difference of opinion among those who have actually seen specimens, as to the standing and relationships of the different genera. With (1915, p. 36) considers that *Heterocalanus* Wolfenden and *Bradycalanus* Scott are synonymous with *Macrocalanus* Sars.

Only one of the various genera assigned to the family was found in the collections from San Francisco Bay.

Genus **Calanus** Leach

Calanus, Giesbrecht, 1892, pp. 45, 88, 725.
Calanus, Giesbrecht and Schmeil, 1898, p. 14.
Calanus, Wheeler, 1899, p. 164.
Calanus, Sars, 1903, p. 8.
Calanus, Esterly, 1905, p. 123.
Calanus, Van Breemen, 1908, p. 6.
Calanus, Scott, 1909, p. 7.
Calanus, Pesta, 1920, p. 493.

Characters.—Appendages of males and females closely alike; only difference to be readily noted is in fifth feet; these of swimming type in both sexes, but slightly asymmetrical in male (fig. A, 9). No hook-like outgrowth on second basal of first foot; third joint of outer ramus of second, third, and fourth feet has two outer marginal spines. Long setae of maxillae not densely plumose.

Calanus finmarchicus Gunner

Figure A

Calanus finmarchicus, Giesbrecht, 1898, pp. 89, 218; pl. 6, fig. 19; pl. 7, figs. 32, 33; pl. 8, figs. 3, 15, 21, 31, 33.
Calanus finmarchicus, Giesbrecht and Schmeil, 1898, p. 14.
Calanus finmarchicus, Wheeler, 1899, p. 164, fig. 1.
Calanus finmarchicus, Sars, 1903, p. 3, pls. 1, 2, 3.
Calanus finmarchicus, Esterly, 1905, p. 125, fig. 1.
Calanus finmarchicus, Van Breemen, 1908, p. 7.
Calanus finmarchicus, With, 1915, p. 10, text-figs. 1–5.
Calanus finmarchicus, Pesta, 1920, p. 493, text-fig. A, 1.

Characters.—Sexes readily distinguished because abdomen of female (fig. A, 2, 6) has 4 segments, that of male 5 (fig. A, 4, 5). Shape of cephalothorax not greatly different in sexes, head and margins of last thoracic segment smoothly rounded (fig. A, 4, 5, 6). Anterior antennae symmetrical in both sexes, female resembling male, but basal joint in male (fig. A, 22) a good deal heavier and broader than in female (fig. A, 1); this difference appears even when appendage is attached to body. Fifth feet of sexes differ noticeably; fifth foot in female essentially similar to second, third, and fourth (fig. A, 15, 8, 11, 13); right and left feet alike. In males, fifth feet asymmetrical (fig. A, 9, 18, 19); right foot much like other pairs but outer ramus lacks bristles on inner margin; outer ramus of left foot one and one-half times length of outer ramus of right foot; first joint of outer ramus of left foot fully as long as first two joints of right outer ramus (fig. A, 9). First and second joints of left outer ramus about equal in length; third joint about half as long as either of others; terminal bristle of third joint slender, straight, as long as, or longer than, the joint; third joint has minute outer marginal spine (fig. A, 18).

In both males and females inner border of first basal of both fifth feet carries row of teeth, curving, at about middle of length of joint, toward outer margin so there is a marked concavity toward inner side (fig. A, 9, 10, 16, 17); in some specimens inner margin itself appears concave (fig. A, 10), in others the row of teeth curves away from margin of joint (fig. A, 17).

Dimensions.—Length of females, 4.5 to 5.3 mm.; of males, 4 to 4.4 mm.

Distribution.—Farran (1910, p. 83) states that the center of distribution of this important species seems to be in the North Atlantic. But it has been reported from the South Atlantic, the west coast of South America, the Mediterranean, the Adriatic, the Red Sea, and the Polar Ocean. Farran (1920) has discussed the distribution of this copepod in the sea southwest of Ireland. He includes under the one name *C. helgolandicus*, *C. septentrionalis*, and the species

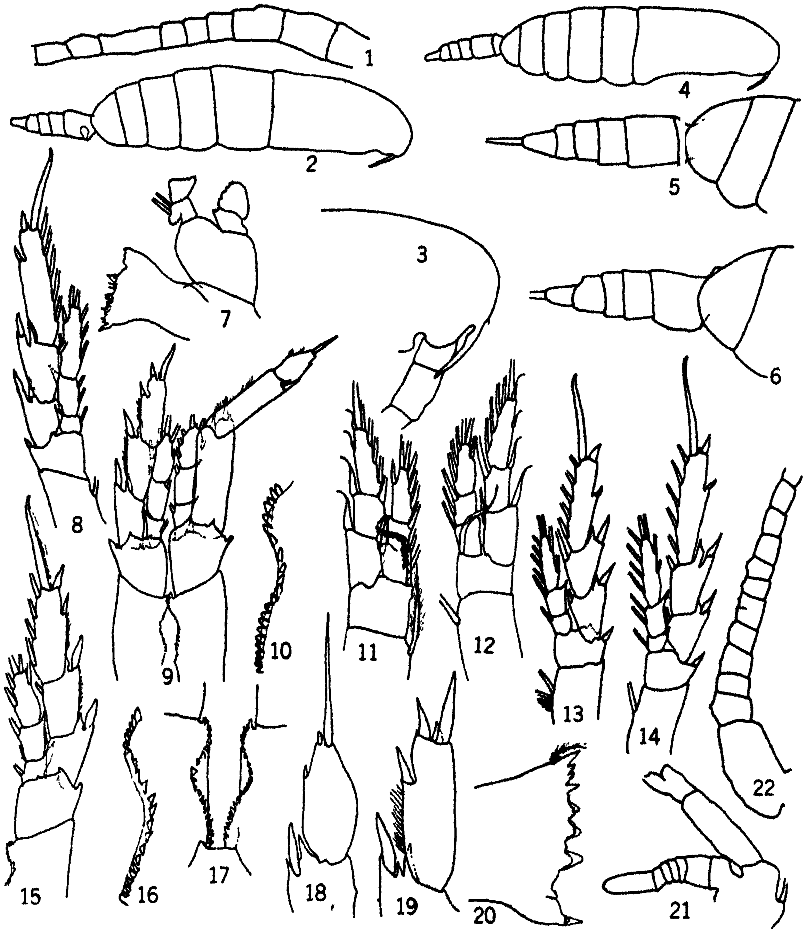

Fig. A. *Calanus finmarchicus*. 1: Base of anterior antenna, female. × 32. 2: Female, from side. × 7. 3: Head of female, from side. × 32. 4: Male, from side. × 7. 5: Abdomen and posterior part of thorax, from side, male. × 32. 6: Abdomen and posterior part of thorax, from side, female. × 32. 7: Mandible, female. × 64. 8: Fourth foot, female. × 48. 9: Fifth pair of feet, male. × 26; left foot is on right of figure. 10: Denticulation on inner margin of first basal of fifth foot, female. × 112. 11: First foot, female. × 52. 12: First foot, male. × 64. 13: Second foot, female. × 48. 14: Third foot, male. × 48. 15: Fifth foot, female. × 52. 16: Denticulation on inner margin of first basal of fifth foot, male. × 256. 17: Denticulation on inner margin of fifth feet, female. × 140. 18: Terminal joint of left fifth foot, male. × 140. 19: Terminal joint of right fifth foot, male. × 140. 20: Blade of mandible, male. × 140. 21: Posterior antenna, female. × 48. 22. First eleven joints of anterior antenna, male. × 32.

known as *C. finmarchicus* in the narrower sense. The species has been reported from American and Canadian waters by Herdman, Thompson, and Scott (1897); from Vineyard Sound near Gay Head by Wheeler (1899, p. 165); from the San Diego region by Esterly (1905); from Narragansett Bay by Williams (1906 and 1907); from Woods Hole at the Fisheries wharf by Sharpe (1910); and from various stations of the Canadian Arctic Expedition by Willey (1920). Some idea of the economic importance of the species is given by Farran (1910, p. 88).

Remarks.—There has been much discussion as to whether *C. finmarchicus* and *C. helgolandicus* are different species, as maintained by Sars. The question has been gone into by With (1915, pp. 12–14), and he seems not to be convinced of any specific difference (see also Pesta, 1920, p. 495). Though I have examined only specimens from the coast of California, the San Diego region chiefly, my own opinion is that the two forms are not specifically different. As With remarks (pp. 13–14) the question whether we are dealing with one species or with two should be settled because of its bearing on biological and distributional studies.

To descriptions of the usual features With has added accounts of the structure of the labium and labrum and of the distribution of the secretory pores. Wheeler (1899, p. 165) and Williams (1906, p. 640) state that their specimens lacked the concave margin on the first basal of the fifth feet.

Family Paracalanidae

The genera in this family are *Paracalanus* Boeck, *Acrocalanus* Giesbrecht, and *Calocalanus* Giesbrecht. The first is the only one found in the material from San Francisco Bay.

Genus **Paracalanus** Boeck

Paracalanus, Giesbrecht, 1892, pp. 48, 164, 757.
Paracalanus, Giesbrecht and Schmeil, 1898, p. 23.
Paracalanus, Wheeler, 1899, p. 168.
Paracalanus, Sars, 1903, p. 17.
Paracalanus, Esterly, 1905, p. 139.
Paracalanus, Van Breemen, 1908, p. 20.
Paracalanus, Scott, 1909, p. 26.
Paracalanus, Pesta, 1920, p. 500.

Characters.—Animals among smallest of free-swimming forms found in bay. Body quite robust; abdomen of female 4-segmented, of male 5-segmented.

Head fused with thorax, and fourth and fifth segments of thorax fused with each other, so cephalothorax composed of 4 visible segments.

Outer rami of first four swimming feet 3-jointed; inner ramus in second, third, and fourth pairs 3-jointed, in first pair 2-jointed. Third joint of outer

ramus of second, third, and fourth pairs has 2 outer marginal spines, and outer margin is denticulate proximal to first spine. Terminal joint of inner ramus of all except first foot has 5 bristles; in first foot, 7 bristles.

Fifth feet of female symmetrical, minute, and 2-jointed, with one free joint. In male, asymmetrical, left fifth foot 5-jointed, three or four times as long as right foot, which is like fifth feet in female.

Remarks.—According to Scott (1909, p. 28) this genus and *Acrocalanus* are closely allied; but in the latter the fifth feet of the female are absent or rudimentary and the males have only the left fifth foot. In *Acrocalanus*, also, the outer margin of the second as well as the third joint of the outer ramus of the third and fourth feet is denticulate. *Paracalanus* is to be easily distinguished from *Calocalanus* because in the latter the abdomen of the female is very short and consists of 2 or 3 segments. In the males of *Calocalanus* the left fifth foot is 5-jointed and considerably longer than the 3-jointed right foot; the fifth feet of the female have 2 or 3 free joints.

Paracalanus parvus Claus

Figure B

Paracalanus parvus, Giesbrecht, 1892, pp. 164, 170; pl. 1, fig. 5; pl. 6, figs. 28–30; pl. 9, figs. 5–11, 25, 27, 31, 32.
Paracalanus parvus, Giesbrecht and Schmeil, 1898, p. 24.
Paracalanus parvus, Wheeler, 1899, p. 168, fig. 6.
Paracalanus parvus, Sars, 1903, p. 17, pls. 8 and 9.
Paracalanus parvus, Esterly, 1905, p. 140, fig. 12.
Paracalanus parvus, Van Breemen, 1908, p. 20, fig. 17.
Paracalanus parvus, Scott, 1909, p. 27.
Paracalanus parvus, With, 1915, p. 54, fig. 12.
Paracalanus parvus, Pesta, 1920, p. 500, text-fig. A, 12.

Characters.—**Head, and sides of last thoracic segment, smoothly rounded (fig. B, 1, 2, 3, 6, 7, 8, 13). Rostrum double, consisting of slender, delicate filaments (fig. B, 2, 3, 6, 13). Abdomen of female 4-segmented (fig. B, 1); relative lengths of segments 9: 4: 4: 6; abdomen of male 5-segmented, relative lengths 9: 18: 7: 12: 15 (fig. B, 5). Anterior antennae of female 25-jointed, of male 20-jointed (fig. B, 14).**

Second joint of inner ramus in second and third feet has row of spines on posterior surface (fig. B, 10, 11); short, rather stout teeth, closely set along outer margin of third joint of exopodite of second, third, and fourth feet, are characteristic (fig. B, 10, 11).

Fifth feet differ greatly in sexes; in female (fig. B, 15), symmetrical, having basal part and 1 free joint, basal stout and broad, free joint more slender with margins nearly straight and about parallel, terminal spine nearly as long as second joint, a very small, short spine at outer side of long one.

Fifth feet of male (fig. B, 16) asymmetrical, left foot four or five times as long as right, with basal and 4 free joints, tip of last joint ending in characteristic double prong; right foot closely resembles fifth feet of female, with basal

and 1 free joint, latter with a long spine and a very short one; exclusive of terminal spine, right foot of male no longer than one of free joints of left foot. In many preserved male specimens left fifth foot is bent sharply toward dorsal side of animal (fig. B, 8).

Dimensions.—Length of female, 1.3 mm.; of male, 1.4 mm.

Distribution.—According to Farran (1910, p. 61), *Paracalanus parvus* is of "almost world-wide distribution." He also states (1920, p. 21) that it is a euryhaline species. It has been found in the North and South Atlantic, Mediter-

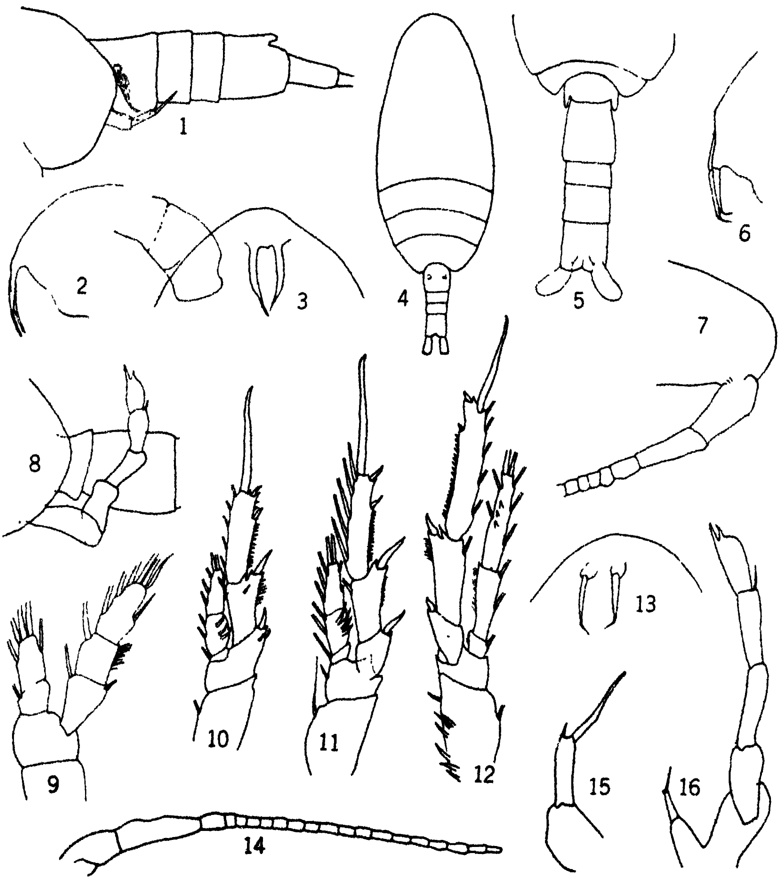

Fig. B. *Paracalanus parvus.* 1: Abdomen and posterior margin of thorax, from side, female. × 140; fifth foot shown under first segment of abdomen. 2: Anterior portion of head, from side, female. × 140. 3: Anterior portion of head, from below, female. × 140. 4: Body, from dorsal side, female. × 26. 5: Abdomen and posterior part of thorax, from ventral side, male. × 64. 6: Anterior portion of head, from side, male. × 140. 7: Anterior portion of head and basal joints of anterior antenna, from side, male. × 64. 8: Posterior border of thorax, first and second segments of abdomen, left fifth foot, from side, male. × 140. 9: First foot, male. × 186. 10: Second foot, female. × 140. 11: Second foot, male. × 140. 12: Fourth foot, female. × 140. 13: Anterior portion of head, from below, male. × 140. 14: Anterior antenna, male. × 64. 15: Fifth foot, female. × 186. 16: Fifth pair of feet, male. × 112.

ranean, Black Sea, Gulf of Suez, Red Sea, Arabian Sea, Indian Ocean, Pacific Ocean. It is regarded as a tropical and temperate form. It has been recorded from American waters, by Wheeler (1899, p. 169) from the Gulf Stream south of Martha's Vineyard; by Esterly (1905) from the San Diego region. It is very abundant at times in surface hauls at the pier of the Scripps Institution at La Jolla.

Family PSEUDOCALANIDAE

With (1915) includes in this family *Pseudocalanus* Boeck, *Microcalanus* Sars, *Clausocalanus* Giesbrecht, and *Spinocalanus* Giesbrecht. Scott (1909) assigns to it *Clausocalanus* Giesbrecht, *Oxycalanus* Farran, and *Monacilla* Sars. According to Giesbrecht (1892, p. 21), *Drepanopus* Brady and *Moebianus* Giesbrecht are to be put in the subfamily Clausocalaninae along with *Pseudocalanus*, *Spinocalanus*, *Ctenocalanus*, and *Clausocalanus*, but Sars (1903, p. 19) followed by Van Breemen (1908) removed *Drepanopus* (= *Stephos* Sars) to another section. Sars (1903, p. 19) has shown why the family should be named from the genus *Pseudocalanus* instead of *Clausocalanus*.

The bay collections contained the genera *Clausocalanus* Giesbrecht and *Ctenocalanus* Giesbrecht.

Genus **Clausocalanus** Giesbrecht

Clausocalanus, Giesbrecht, 1892, pp. 50, 185, 733.
Clausocalanus, Giesbrecht and Schmeil, 1898, p. 27.
Clausocalanus, Wheeler, 1899, p. 171.
Clausocalanus, Esterly, 1905, p. 142.
Clausocalanus, Van Breemen, 1908, p. 22.
Clausocalanus, Scott, 1909, p. 31.
Clausocalanus, Pesta, 1920, p. 505.

Characters.—Head fused with thorax, fourth thoracic segment with fifth, so cephalothorax has 4 visible segments. Abdomen of female 4-segmented, of male 5-segmented. Rostrum in female bifid, rather short and stout; in male reduced to slight elevation. Anterior antennae in female 23-jointed, in male 17-jointed.

First four pairs of feet with 3-jointed outer ramus; inner ramus of first foot 1-jointed, of second 2-jointed, of third and fourth 3-jointed. Terminal joint of outer ramus in all but first foot with 3 outer marginal spines and 3 inner marginal setae. Pronounced notching of distal margin of second basal of second and third feet is characteristic of genus.

Fifth feet present in both sexes; in female, symmetrical, minute, with a basal and 2 free joints, no bristles or spines; in male, asymmetrical, left foot much elongated, 5-jointed; right foot 1- or 3-jointed, about one-fifth as long as left.

Remarks.—It is not easy to distinguish specimens of *Clausocalanus* from *Paracalanus*, but it is less difficult if the rostrum can be seen.

Clausocalanus arcuicornis Dana

Figure C

Clausocalanus arcuicornis, Giesbrecht, 1892, pp. 186, 193; pl. 1, fig. 14; pl. 2, fig. 7; pl. 10, figs. 3–8, 14, 16, 17, 19; pl. 36, figs. 29–31, 34.

Clausocalanus arcuicornis, Giesbrecht and Schmeil, 1898, p. 27.

Clausocalanus arcuicornis, Wheeler, 1899, p. 171, fig. 9.

Clausocalanus arcuicornis, Esterly, 1905, p. 142, fig. 13.

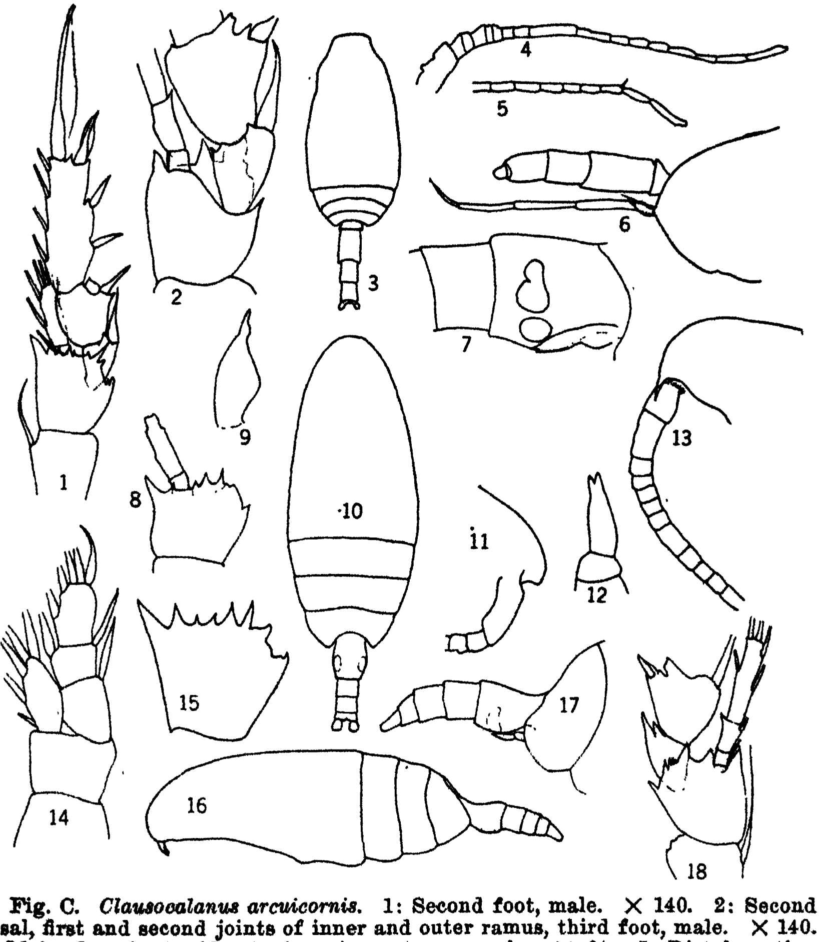

Fig. C. *Clausocalanus arcuicornis*. 1: Second foot, male. × 140. 2: Second basal, first and second joints of inner and outer ramus, third foot, male. × 140. 3: Male, dorsal. × 32. 4: Anterior antenna, male. × 64. 5: Distal portion of anterior antenna, female. × 64. 6: Abdomen and posterior part of thorax, from side, male. × 64; fifth feet also shown, the longer one being the left. 7: First and second segments of abdomen, fifth foot, obliquely from right side, female. × 140. 8: Second basal and inner ramus, second foot, female. × 140. 9: Right fifth foot, from right side, male. × 282. 10: Female, dorsal. × 34. 11: Head of male, from side. × 64. 12: Fifth foot, female. × 282. 13: Head and part of anterior antenna, from side, female. × 64. 14: First foot, female. × 166. 15: Second basal of second foot, female. × 166. 16: Female, from side. × 34. 17: Abdomen and last segment of thorax, from side, female. × 64. 18: Basals, inner ramus, first two joints of outer ramus, third foot, female. × 140.

Clausocalanus arcuicornis, Van Breemen, 1908, p. 23, fig. 20.
Clausocalanus arcuicornis, Scott, 1909, p. 32.
Clausocalanus arcuicornis, With, 1915, p. 68, pl. 1, figs. 9*a*–9*d* (see note).
Clausocalanus arcuicornis, Pesta, 1920, p. 505, text-fig. B, 14.

NOTE.—With has used the specific name *arcuiformis* throughout.

Characters.—Head. seen from side, rounded in both sexes (fig. C, 11, 13, 16); in dorsal view head of male truncate (fig. C, 3) compared to smooth curve in female (fig. C, 10). Rostrum of female consists of 2 rather heavy prongs (fig. C, 13, 16), but in male only a slight elevation (fig. C, 11).

Cephalothorax of both sexes has 4 visible segments (male, fig. C, 3, 6; female, fig. C, 10, 16); relative lengths in male, 50: 4: 4: 3, in female 40: 15: 10: 8. Abdomen of female 4-segmented, relative lengths 15: 6: 6: 3; in male, 5-segmented, relative lengths 3: 11: 8: 6: 1. Furca about as long as wide.

Anterior antennae symmetrical in both sexes; 23-jointed in female, 17-jointed in male. Distal margin of second basal in second and third feet toothed and notched in both sexes (fig. C, 1, 2, 8, 15, 18).

Fifth feet sharply distinguish sexes; in female symmetrical, uniramous, 3-jointed, terminal joint notched at distal end and about as long as others together (fig. C, 7, 12, 17). In male asymmetrical, right 3-jointed, not over half as long as first joint of left; left 5-jointed, when extended backwards reaching beyond end of body (fig. C, 6, 9).

Dimensions.—Length of female, 1.38 mm.; of male, 1.2 mm.

Distribution.—According to Farran (1910, p. 91), this copepod is characteristic of warm water, oceanic areas. It has been recorded from the Atlantic, Pacific, and Indian oceans, the Mediterranean and Red seas. Farran (1920, p. 24) states that *Clausocalanus arcuicornis* is one of the oceanic species of such regular occurrence that they "might almost be used to measure the average salinity." Wheeler (1899, p. 171) obtained specimens from the Gulf Stream in July. This copepod occurs in the San Diego region (Esterly, 1905) and sometimes it is found in swarms at the pier at La Jolla.

Remarks.—This species of *Clausocalanus* is to be distinguished from *C. furcatus* Brady in that the latter has the genital segment shorter than either of the two succeeding segments, and the furca is twice as long as broad.

Genus **Ctenocalanus** Giesbrecht

Ctenocalanus, Giesbrecht, 1892, pp. 50, 194.
Ctenocalanus, Giesbrecht and Schmeil, 1898, p. 28.
Ctenocalanus, Van Breemen, 1908, p. 24.
Ctenocalanus, Pesta, 1920, p. 506.

Characters.—Resembles *Clausocalanus*, but rostrum consists of two delicate, slender filaments; outer marginal spines of third joint of outer ramus in third and fourth feet toothed along inner margin; distal borders of second basal of second and third feet not toothed; fifth foot only on left side, of variable form in female according to Giesbrecht (1892, p. 196); in male fifth foot longer and more slender, 5-jointed.

Remarks.—There is but one species of the genus, and though the specimens from the bay do not conform entirely to Giesbrecht's account, the differences do not seem to be of specific rank.

Ctenocalanus vanus Giesbrecht

Figures D and E

Ctenocalanus vanus, Giesbrecht, 1892, p. 194; pl. 10, figs. 20, 21, 26; pl. 36, fig. 28.

Ctenocalanus vanus (male), Wolfenden, 1904, p. 124; pl. 9, fig. 9.

Ctenocalanus vanus, Van Breemen, 1908, p. 24, fig. 21.

Ctenocalanus vanus, Pesta, 1920, text-fig. B, 16.

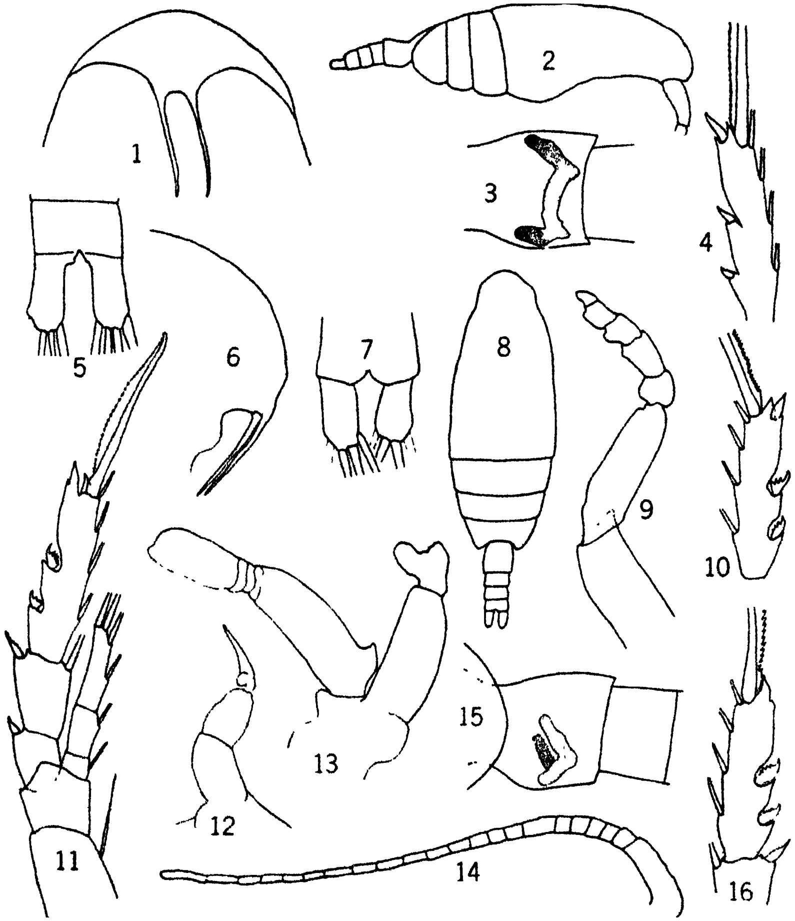

Fig. D. *Ctenocalanus vanus*, female. 1: Anterior part of head, from below. × 140. 2: Entire animal, from side. × 32. 3: Genital segment with seminal receptacle, from below. × 140. 4: Third joint of outer ramus of third foot. × 140. 5: Furca and last segment of abdomen, of a specimen from La Jolla. × 140. 6: Anterior part of head, from side. × 140. 7: Furca. × 140. 8: Entire animal, dorsal. × 32. 9: Posterior maxilliped. × 140. 10: Third joint of outer ramus of third foot. × 140; a different specimen than in 4. 11: Third foot. × 140; from a third animal (cf. figs. 4 and 10). 12: Fifth foot of a specimen from La Jolla. × 282. 13: Posterior antenna. × 140. 14: Anterior antenna. × 48. 15: First two segments of abdomen, from side. × 140. 16: Third joint of outer ramus of fourth foot. × 140.

Characters.—Head, and sides of last thoracic segment, rounded in both sexes (fig. D, 2, 5, 8, 15; fig. E, 1, 2, 3, 8). Rostrum of two delicate, slender filaments (fig. D, 1, 6; fig. E, 3, 6).

Cephalothorax has 4 visible segments, relative lengths in both sexes 55: 6: 9: 4. Abdomen of female 4-segmented, relative lengths 8: 4: 4: 3; of male 5-segmented, relative lengths 3: 10: 7: 5: 3; furcal rami about twice as long as broad, outer marginal seta lacking (fig. D, 7; fig. E, 9).

Anterior antennae of female 22-jointed (fig. D, 14) (according to Giesbrecht, 1892, p. 195, 24-jointed); 21-jointed in male (fig. E, 11). Outer ramus of posterior antennae longer than inner, in female outer is half again as long as inner, in male over twice as long (fig. D, 13; fig. E, 12).

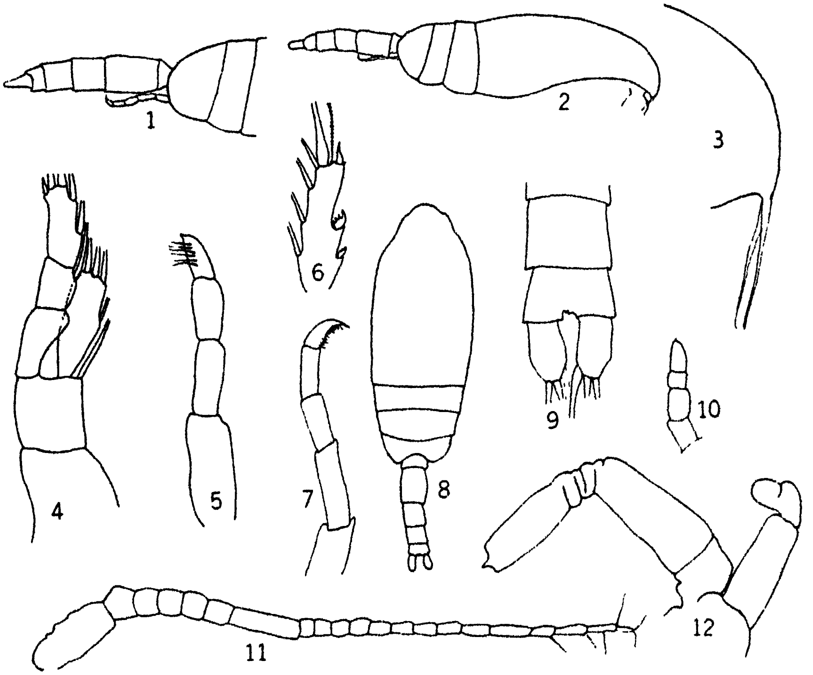

Fig. E. *Ctenocalanus vanus*, male. 1: Abdomen and last two segments of thorax, from side. × 48. 2: Entire animal, from side. × 32. 3: Anterior part of head, from side. × 140. 4: First foot. × 96. 5: Fifth foot. × 282; specimen was immature. 6: Third joint of outer ramus of third foot. × 140. 7: Fifth foot. × 96. 8: Entire animal from dorsal side. × 32. 9: Furca and last two segments of abdomen, from ventral side. × 140. 10: Fifth foot of immature specimen. × 140. 11: Anterior antenna. × 32. 12: Posterior antenna. × 140.

Swimming feet similar in sexes; notable that outer spines of end joint of outer ramus of third and fourth feet are ctenate (fig. D, 10, 11, 16; fig. E, 6), though in one female outer spines were of usual form (fig. D, 4).

Fifth foot of female from bay unknown, but in specimen from La Jolla, California, is found only on left side, 2-jointed, heavy, awl-like structure at end (fig. D, 12); in male, only left foot present, 5-jointed, terminal joint ending in sharp spur, fringe of bristles on inner margin of last joint (fig. E, 1, 2, 7). According to Wolfenden (1904, p. 124) right fifth foot represented by very short stump.

Dimensions.—Length of female, 1.21 mm.; of male, 1.36 mm.

Distribution.—*Ctenocalanus* has been recorded from the Atlantic, Mediterranean, Pacific, and Antarctic. Farran (1920, pp. 16–17) lists it among the oceanic species, and states (p. 24) that like *Clausocalanus* it might almost be used to measure average salinity. So far as I know this copepod has not been recorded before from American waters. It was rare in the collections from the bay, but it occurs at certain times in larger numbers at the pier at La Jolla, where some surface hauls have taken dozens of the animals.

Family TEMORIDAE

Eurytemora Giesbrecht is the only genus of the family that was found in the collections from the bay.

Genus **Eurytemora** Giesbrecht

Eurytemora, Giesbrecht and Schmeil, 1898, p. 102.
Eurytemora, Sars, 1903, p. 99.
Eurytemora, Williams, 1906, p. 645.
Eurytemora, Van Breemen, 1908, p. 98.

Characters.—Head not fused with the first thoracic segment nor fourth segment of thorax with fifth, so cephalothorax has 6 visible segments. Rostrum bifid, consists of small, soft lappets. Abdomen of female 3-segmented, of male 5-segmented, furca symmetrical in both sexes.

Anterior antennae of female symmetrical, 24-jointed; right antenna of male distinctly geniculate, forming grasping organ. First 4 pairs of feet have 3-jointed outer ramus, terminal joint in second, third, and fourth with 2 outer marginal spines; inner ramus of first foot 1-jointed, of second, third, and fourth 2-jointed.

Fifth feet in female symmetrical, 4-jointed, uniramous, penultimate joint with strong projection from inner margin; in male asymmetrical, left 4-jointed, right 4- or 5-jointed, both incurved, end joint of left foot broadened, of right claw-shaped.

Eurytemora hirundoides (Nordquist)

Figures F and G

Eurytemora affinis var. *hirundoides,* Giesbrecht and Schmeil, 1898, p. 104.
Eurytemora hirundoides, Sars, 1903, p. 102, pl. 69.
Eurytemora hirundoides, Van Breemen, 1908, p. 101, fig. 117.
Eurytemora hirundoides, Sharp, 1910, p. 411, fig. 3.

Characters.—Head rounded in both sexes (fig. F, 2, 3; fig. G, 1), rostrum of two soft, sharply pointed outgrowths (fig. F, 7). Last segment of thorax of female expands into pointed, triangular wing extending laterally and backwards (fig. F, 13); in male, thorax rounded behind (fig. G, 1); relative lengths of segments of cephalothorax along mid-dorsal line, in female 32: 10: 12: 9: 6: 12, in male, 30: 10: 8: 7: 5: 1; 'wings' of female extend back at least as far as posterior border of first segment of abdomen, in some as far as middle of second segment (fig. F, 2, 5).

Abdomen of female 3-segmented, relative lengths (with furca) 14: 7: 9: 18; of male 5-segmented, relative lengths (with furca) 5: 8: 6: 4: 7: 18. First segment in both sexes expanded in middle (fig. F, 5, 9); last segment in female densely set with fine, spine-like hairs on dorsal surface near lateral margins and on margins (fig. F, 5, 9). Furcal blades of female 6 times as long as wide; in male 8 times.

Anterior antennae of female 24-jointed, reaching about to posterior border of fourth thoracic segment when reflexed; right antenna of male a grasping organ (fig. G, 4, 8), 2-jointed distal to geniculation, antennae, when folded against body, reaching to second segment of abdomen.

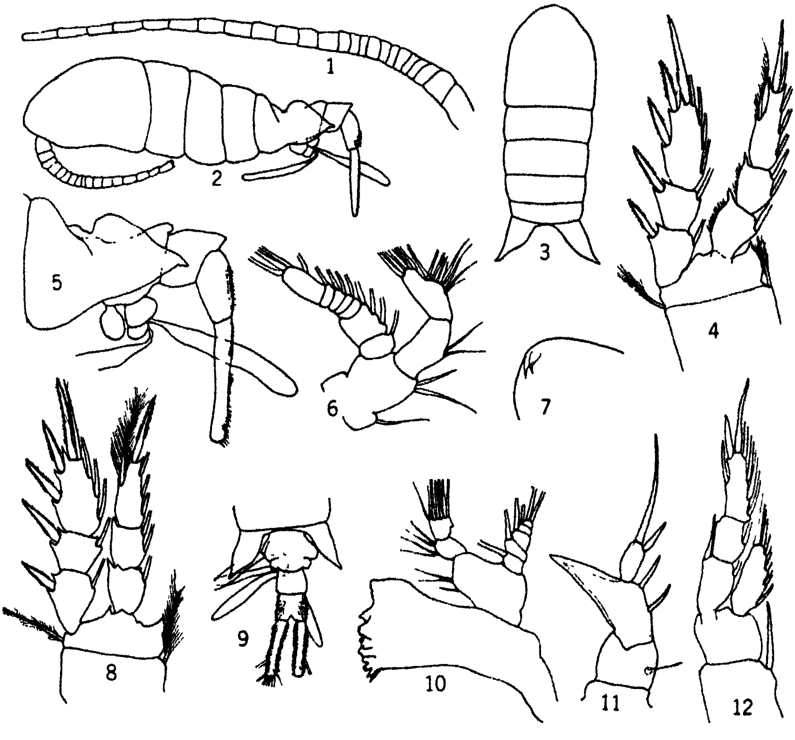

Fig. F. *Eurytemora hirundoides*, female. 1: Anterior antenna. × 64. 2: Entire animal, from side. × 32. 3: Cephalothorax, from dorsal side. × 32. 4: Second foot. × 140. 5: Abdomen and last segment of thorax, from side. × 64. 6: Posterior antenna. × 140. 7: Anterior part of head, obliquely from below. × 64. 8: Third foot. × 140. 9: Abdomen and last segment of thorax, from dorsal side. × 32. 10: Mandible. × 140. 11: Fifth foot. × 140. 12: First foot. × 140.

Fifth feet of female (fig. F, 11) with 2-jointed basal part and 2-jointed exopodite, first joint of which has 2 spines on outer margin and strong, pointed process inside; second joint smaller, long, stiff bristle at tip, shorter spine on outer margin near base of long bristle. Fifth feet of male (fig. G, 9) with 2-jointed exopodites, terminal joint of right slender, dilated near base; end joint of left foot ending in three lobes or blunt processes with concavity between them.

Dimensions.—Length of female, 1.56 mm., of male 1.15 mm. Cephalothorax of a mutilated female was 1.15 mm. to end of wings; broken bodies of two males each 1.06 mm. to end of last segment of abdomen.

Distribution.—The occurrence of *Eurytemora affinis* has been discussed by Scott (1910, p. 112); he states that he includes the "varieties *hispida* and *hirundoides.*" *E. affinis* and its varieties have been recorded from France, the north of Germany, Gulf of Finland (Ostenfeld, 1906, p. 87), the Baltic, British Islands, the greater part of the Norwegian coast, mouth of Volga in Caspian Sea, Gulf of St. Lawrence, Woods Hole (Sharpe, 1910, p. 411), Narragansett Bay, and Charlestown Pond (Williams, 1906, p. 647; 1907, p. 71). It has not been recorded previously from the Pacific Coast, but Willey (1920) found several other species of the genus in the collections of the Canadian Arctic Expedition.

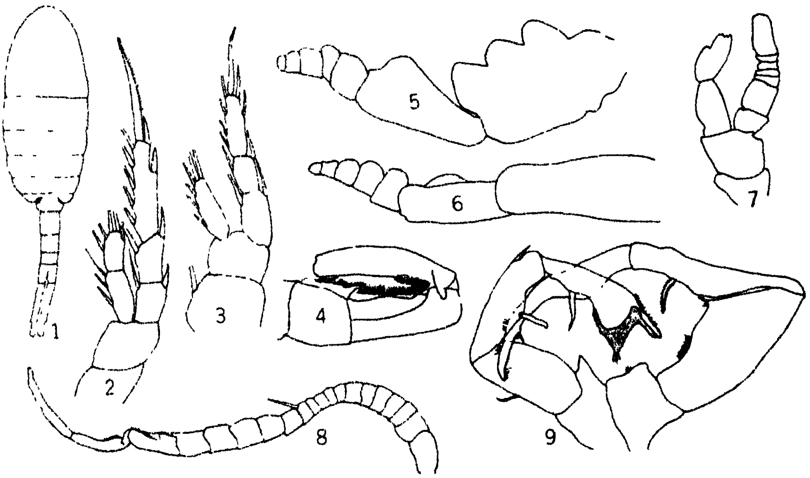

Fig. G. *Eurytemora hirundoides*, male. 1: Entire animal, dorsal. × 32. 2: Fourth foot. × 140. 3: First foot. × 140. 4: Two joints of anterior antenna proximal to geniculation, and the one distal to it. × 140. 5: Anterior maxilliped. × 282. 6: Posterior maxilliped. × 282. 7: Posterior antenna. × 140. 8: Right anterior antenna. × 64. 9: Fifth pair of feet. × 140; left foot at left of drawing.

Remarks.—According to Giesbrecht and Schmeil, this copepod is probably the pelagic form of *E. affinis* (1898, p. 104), but Sars (1903, p. 102) considers *E. affinis* and *E. hirundoides* to be distinct species, and in this view he was followed by Van Breemen (1908).

According to Scott (1910, p. 114). *E. hirundoides* (or *E. affinis* var. *hirundoides*), in relation to hydrographic conditions, is intermediate between fresh-water forms and truly marine ones and can accommodate itself to conditions where the salinity may approach either extreme. Most of the bay specimens were found where the salinity was the lowest encountered in the survey.

Family METRIDIIDAE

Of the two genera, *Metridia* Boeck and *Pleuromamma* Giesbrecht, which comprise the family, only the former was found in the bay. These genera are much alike; the latter has a conspicuous brown or black knob on the side of the first thoracic segment, while *Metridia* does not.

Genus **Metridia** Boeck

Metridia, Giesbrecht, 1892, pp. 61, 339, 749.
Metridia, Giesbrecht and Schmeil, 1898, p. 106.
Metridia, Wheeler, 1899, p. 175.
Metridia, Sars, 1903, p. 110.
Metridia, Esterly, 1905, p. 177.
Metridia, Scott, 1909, p. 120.

Characters.—Cephalothorax with 5 visible segments; abdomen symmetrical in both sexes, 3-segmented in female, 5-segmented in male. Rostrum double, consisting of slender, delicate filaments. Anterior antennae 23-jointed in female; in male, one antenna, usually the left, is of grasping type.

Rami of first four feet 3-jointed, end joint of outer ramus with 3 outer spines; first joint of inner ramus of second foot strongly hooked on inner margin, joint narrower proximal to hooks than elsewhere (fig. H, 10, 14).

Fifth feet in female small, 3- or 4-jointed, terminal joint with slender bristles; in male, 5-jointed, right foot heavier and longer than left, third joint of right, in most species, with long, stout spine on inner margin directed toward tip of foot and reaching as far as middle of last joint.

Metridia sp.

Figure H

Characters.—There can be little doubt that the species is either *M. lucens* Boeck or *M. boecki* Giesbrecht; certain recognition depends on fifth feet of female. Last thoracic segment with slightly pointed margin in both sexes (fig. H, 3, 4, 6, 8); furca longer than anal segment (fig. H, 7, 13). Fifth feet of males of form characteristic of genus (fig. H, 15).

Dimensions.—Length of female, 2.6 mm.; of male, 2.1 mm.

Remarks.—According to Scott (1909, p. 120) the rami of the furca in *M. boecki* are as long as the anal segment. and the fifth foot has 3 free joints while in *lucens* there are 2. Van Breemen (1908) states that the furca of *lucens* is not so long as the anal segment, and Sars (1903, p. 103) says that in *lucens* the furca "is scarcely as long as the anal segment." According to Giesbrecht (1892, pp. 345, 346), the furca of *M. hibernica* (= *lucens*) is somewhat shorter than the last segment of the abdomen, and the furca of *boecki* is as long as the segment. If the fifth feet of females similar to those already found should conform to the *boecki* type, the San Francisco species should be set down as *M. boecki.*

Family LUCICUTIIDAE

Genus **Lucicutia** Giesbrecht

Leuckartia, Giesbrecht, 1892, pp. 62, 358.
Lucicutia, Giesbrecht and Schmeil, 1898, p. 110.
Lucicutia, Esterly, 1905, p. 179.
Lucicutia, Van Breemen, 1908, p. 111.
Lucicutia, Scott, 1909, p. 125.
Lucicutia, Pesta, 1920, p. 529.

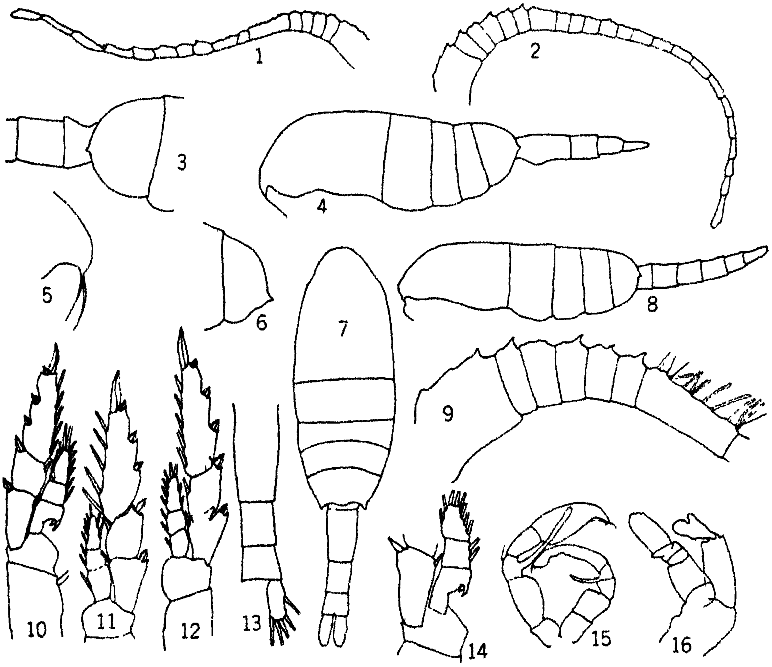

Fig. H. *Metridia* sp. 1: Anterior antenna, male. × 32. 2: Anterior antenna, female. × 32. 3: Last segment of thorax and first two segments of abdomen, male. × 64. 4: Entire animal, from side, female. × 24. 5: Anterior part of head, from side, male. × 64. 6: Last segment of thorax, from side, female. × 32; from a second specimen. 7: Entire animal, dorsal, female. × 20. 8: Entire animal, from side, male. × 26. 9: Basal portion of anterior antenna, female. × 64. 10: Second foot, female. × 64. 11: Third foot, male. × 64. 12: Third foot, female. × 64. 13: Abdomen and furca, dorsal, female. × 32; a second specimen. 14: Second basal, first joint of outer ramus, entire inner ramus, female. × 64; the foot opposite to one shown in 10. 15: Fifth pair of feet, male. × 64; right foot at left of drawing. 16: Posterior antenna, male. × 64.

Characters.—Head broad as seen from above, not fused with thorax, but fourth and fifth thoracic segments fused, so cephalothorax has 5 visible segments. Rostral filaments slender and delicate. Abdomen of female 4-segmented, symmetrical in most species; of male 5-segmented. Furca always symmetrical.

Anterior antennae of female 24-jointed, symmetrical; in male, left antenna a grasping organ, geniculation between joints 18 and 19. Outer lobe of maxilla with but 5 bristles.

Outer rami of first four pairs of feet 3-jointed; inner rami of all but first feet 3-jointed, of first foot 2- or 3-jointed. Fifth feet of female symmetrical, rami 3-jointed, inner margin of second joint of outer ramus with characteristic, curved, awl-shaped spine on inner margin (fig. I, 9). Fifth feet of male asymmetrical, rami of left 3-jointed, of right 2-jointed; terminal joint of right foot claw-like, opposable to proximal joints to form grasping organ.

Remarks.—One female was found in the "Sausalito" haul. The specimen was rather badly torn, and the anterior antennae were broken off at the tenth joint.

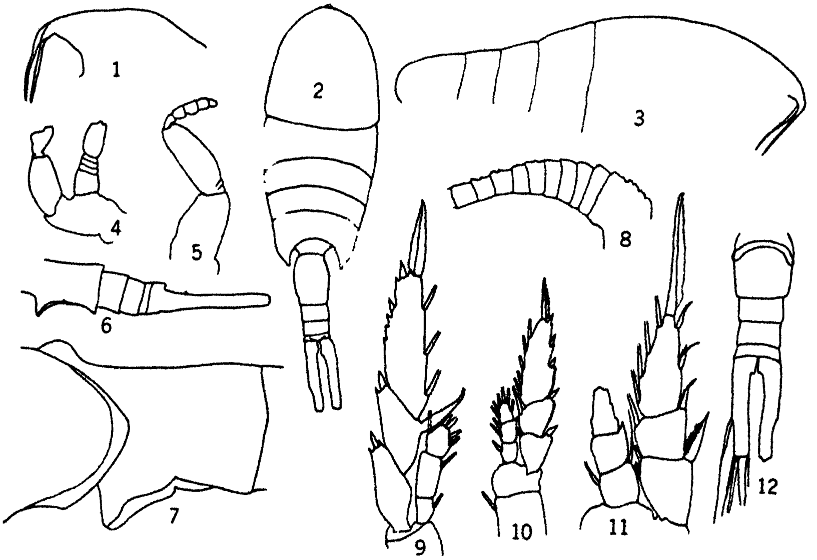

Fig. I. *Lucicutia flavicornis*, female. 1: Anterior portion of head. × 140. 2: Entire animal, dorsal. × 32; torn portions are indicated by dotted lines. 3: Outline of dorsal part of cephalothorax, from side. × 48. 4: Posterior antenna. × 64. 5: Posterior maxilliped. × 64. 6: Abdomen and furca, from side. × 48. 7: Posterior margin of last segment of thorax, genital segment, from side. × 140. 8: Basal part of anterior antenna. × 64. 9: Fifth foot. × 140. 10: Second foot. × 64. 11: First foot. × 140. 12: Abdomen and furca, ventral. × 48.

Lucicutia flavicornis (Claus)

Figure I

Leuckartia flavicornis, Giesbrecht, 1892, p. 358, pl. 5, fig. 4; pl. 19, figs. 2, 3, 15, 17, 21, 23, 29, 38; pl. 38, figs. 38, 40.

Lucicutia flavicornis, Giesbrecht and Schmeil, 1898, p. 111.

Lucicutia flavicornis, Esterly, 1905, p. 180, fig. 36.

Lucicutia flavicornis, Van Breemen, 1908, p. 112, fig. 129.

Lucicutia flavicornis, Scott, 1909, p. 125.

Lucicutia flavicornis, Pesta, 1920, p. 530, text-fig. D, 42.

Characters.—Head broadly rounded in front as seen from above, delicate rostral filaments on a base which projects beyond forehead as little bead (fig. I, 2). Posterior margin of thorax rounded, with backward continuation, like a lamella, which does not extend completely around margin (fig. I, 7). Cephalothorax twice as long in dorsal line as greatest width, one and one-half times length of abdomen and furca; relative lengths of segments of cephalothorax 40: 10: 9: 8: 9. Abdomen 4-segmented, symmetrical, relative lengths of segments and furca 17: 5: 4: 2: 24 (fig. I, 2, 6); genital segment protrudes on ventral side into scoop-shaped process which is concave on posterior face (fig. I, 6, 7). Furcal rami 5 times as long as width at base, 12 times length of anal segment, nearly as long as combined lengths of first three segments of abdomen. (Scott, 1909, p. 126, states furcal length equals second, third and fourth segments together.) Anterior antennae (according to Giesbrecht, 1892, p. 361) 25-jointed, extend to middle of furca. Rami of posterior antennae of equal lengths (fig. I, 4).

Inner ramus of first foot 3-jointed (fig. I, 11); terminal bristle of outer ramus second to fourth feet not over half length of third joint (fig. I, 10); inner ramus of fifth foot extends to distal border of second joint of outer ramus (fig. I, 9) which has conspicuous, awl-shaped bristle at distal end of inner margin. Outer margin of third joint of outer ramus fifth foot with 3 teeth proximal to first outer spine; first joint of outer ramus about half length of third joint, which is twice length of terminal bristle.

Dimensions.—Length, 2.2 mm.

Distribution.—The species is widely distributed. It has been found in the Atlantic and Pacific, Mediterranean and Red Sea, and in the Indian Ocean. This record from San Francisco Bay and the occurrence in the San Diego region are the only reports that I know of from American waters.

Family PONTELLIDAE

Genus **Paralabidocera** McMurrich

Paralabidocera amphitrites McMurrich

Figures J and K

Paralabidocera amphitrites, McMurrich, 1916, pp. 82–87, figs. 8–14.
Paralabidocera amphitrites, Willey, 1920, pp. 16*k*–20*k*, figs. 14–24.

Characters.—Head in both sexes with prominent hook on each side behind eyes (fig. J, 6; fig. K, 2); rostrum strong, bifid, sharply pointed, recurved (fig. J, 5; fig. K, 1). Last segment of thorax in female with asymmetrical lateral wings or lobes (fig. J, 1, 2); in many individuals lobes are rounded, in others there is a minute projection which has probably been worn off in specimens with rounded lobes (Willey, 1920, p. 17*k*). Last segment of thorax in male much prolonged on right into sharp, heavy spine; left side pointed, but shorter, not ending in long spine (fig. K, 2, 9, 10).

Abdomen of female 3-segmented, first segment always prolonged on left into wing projecting backwards and ending in sharp, spine-like process (fig. J, 1, 2, 3, 4); backward-pointing, keel-shaped outgrowths on ventral side of first segment (fig. J, 3, 4); second lacks conspicuous outgrowths, but prolonged slightly at right, posterior angle. Furca symmetrical. Relative lengths of segments and furca 35: 20: 6: 12. Abdomen of male 5-segmented, only the first asymmetrical, longer on right side (fig. K, 2), a notch in left margin; relative lengths of segments and furca 18: 12: 12: 6: 4: 15.

Anterior antennae of female 24-jointed (fig. J, 14), extending to end of thorax. In male, right antenna of grasping type (fig. K, 6), much swollen in middle, part beyond geniculation 3-jointed, a conspicuous toothed flange standing up on joint 17. Rami of posterior antennae of equal lengths. Jaw of mandible with 5 teeth, ventral the largest (fig. J, 8). Posterior maxilliped

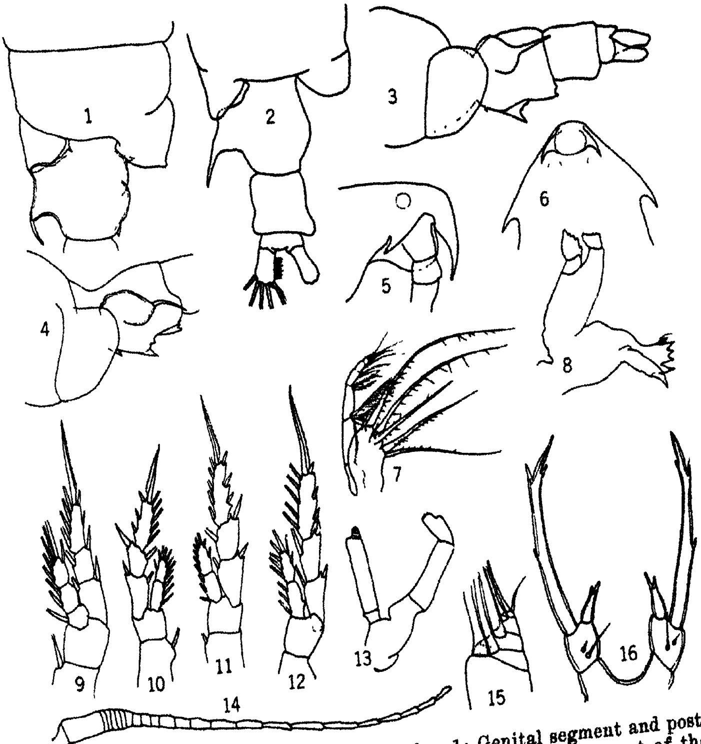

Fig. J. *Paralabidocera amphitrites*, female. 1: Genital segment and posterior part of thorax, dorsal. × 32. 2: Abdomen and furca, posterior part of thorax, dorsal. × 32; a second specimen. 3: Abdomen and furca, posterior part of thorax, from side. × 32. 4: Genital segment and posterior part of thorax, from side. × 32; same specimen as figure 1. 5: Anterior part of head, base of anterior antenna, from side. × 32. 6: Anterior part of head, from below. × 32. 7: Posterior maxilliped. × 48. 8: Mandible. × 48. 9: First foot. × 48. 10: Second foot. × 32. 11: Third foot. × 32. 12: Fourth foot. × 32. 13: Posterior antenna. × 32. 14: Anterior antenna. × 20. 15: Terminal part of outer ramus of posterior antenna. × 140. 16: Fifth pair of feet. × 34.

(fig. J, 7) 7-jointed; according to Willey (1920, pp. 18*k*–19*k*) fifth joint of inner ramus has 3 setae, fourth 1, third 1, second 1, first 2, second basal 3, distal lobe of first basal 1 very heavy, spinose bristle and 2 tiny setae. This is true for many specimens from bay but different individuals vary in number of bristles, as do appendages on two sides of same animal. Middle lobe of first basal with 1 tiny seta in addition to heavy bristles, in our specimens. Outer ramus of first four feet 3-jointed (fig. J, 9–12); inner ramus of first foot 3-jointed (fig. J, 9), of second to fourth 2-jointed.

Fifth feet in female symmetrical (fig. J, 16), first basals meet across middle line, second basal with 2 setae on posterior surface, outer ramus 5 times length of inner. In male fifth feet not symmetrical (fig. K, 3, 4), right foot ends in broad, round hand (fig. K, 3, 14); left foot ends in 3 long, stout spines; on anterior surface of end joint an oval, depressed area, with tufts of delicate hairs (fig. K, 12, 13), outer marginal bristle at middle of length of depression; a raised lobe on proximal border of second basal (fig. K, 10, 11) directed toward protrusion on first basal of right foot.

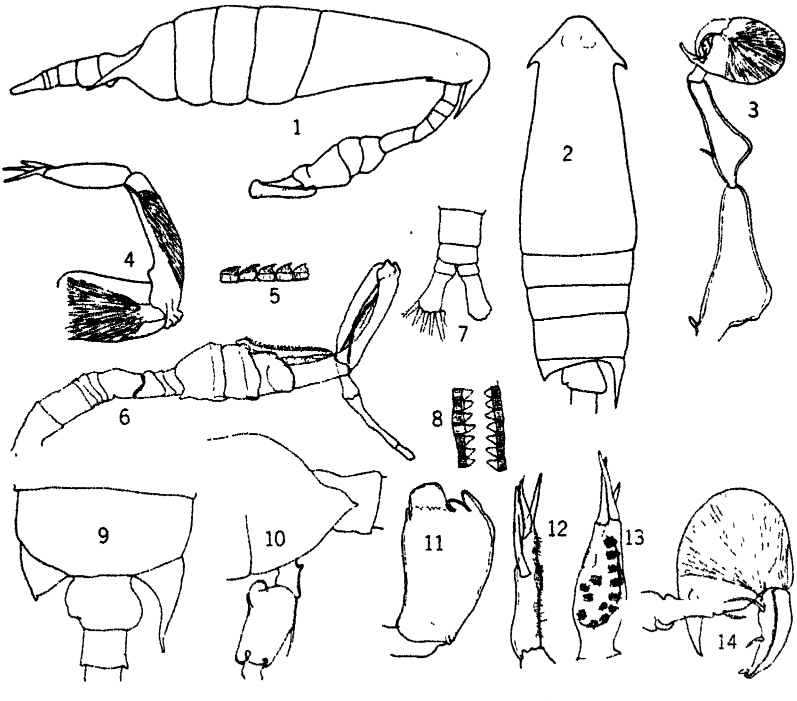

Fig. K. *Paralabidocera amphitrites*, male. 1: Entire animal, from side. × 20. 2: Cephalothorax and genital segment, dorsal. × 20. 3: Right fifth foot. × 34. 4: Left fifth foot. × 34. 5: Some of the teeth on raised flange on thickened middle part of grasping antenna. × 140. 6: The right anterior antenna. × 32. 7: Furca and three posterior segments of abdomen, dorsal. × 32. 8: Some of the teeth on the joints proximal and distal to geniculation of grasping antenna. × 186; proximal teeth are at left. 9: First and second segments of abdomen, posterior part of thorax, dorsal. × 64. 10: Posterior part of thorax; genital segment; basal joints of left fifth foot; all from side. × 32. 11: Second basal of left fifth foot, from side. × 64. 12, 13: Distal joint of left fifth foot. × 64. 14: "Hand" of right fifth foot. × 68.

Dimensions.—Length of female (average), 3.32 mm.; of male (average), 3.00 mm.

Distribution.—McMurrich (1916) and Willey (1920) reported this copepod from the coast of British Columbia and from the collections of the Canadian Arctic Expedition, but the numbers of animals obtained in those regions were small compared to the swarms found in the bay.

Remarks.—This new genus and species was described first in 1916, but in 1920 Willey gave a more complete account based on mature animals. This species appeared in the bay more frequently and in far greater numbers than any of the others obtained there.

Paralabidocera resembles *Pontella* Dana and *Labidocera* Lubbock. Perhaps the distinguishing feature most readily noted is that the grasping antenna of the male has three joints distal to the geniculation, while in *Labidocera* there are four and in *Pontella* two. In Labidocera the first foot has a 2-jointed inner ramus.

Family ACARTIIDAE

Genus **Acartia** Dana

Acartia, Giesbrecht, 1892, pp. 75, 506, 721.
Acartia, Giesbrecht and Schmeil, 1898, p. 150.
Acartia, Wheeler, 1899, p. 182.
Acartia, Sars, 1903, p. 148.
Acartia, Esterly, 1905, p. 203.
Acartia, Van Breemen, 1908, p. 155.
Acartia, Scott, 1909, p. 186.
Acartia, Steuer, 1915.
Acartia, Pesta, 1920, p. 545.

Characters.—Body slender compared with robust forms like *Paracalanus;* no species with lateral hooks on head, none with eye lenses; rostrum lacking in some species, in others two slender filaments. Cephalothorax symmetrical, 5-segmented, fourth segment fused with fifth; posterior margin of thorax rounded in most species, pointed in some. Abdomen symmetrical, 3-segmented in female, 5-segmented in male.

Anterior antennae in female symmetrical, 17-segmented, of nearly uniform diameter for entire length; in male, right antenna of grasping type, middle portion only slightly thickened. Outer ramus of posterior antenna (fig. L, 6; fig. N, 16) very much shorter than inner. Cutting edge of mandible not strongly toothed. Rami of maxilla somewhat retrograded but setae unusually long, curved, and plumose. First maxilliped short, spines long and curved; second with only 4 joints.

Outer rami of first to fourth feet 3-jointed, inner rami 2-jointed; fifth feet in female small, symmetrical, usually uniramous, though some species have inner ramus (Steuer, 1915, p. 394); terminal joint of foot stylet-shaped, more slender in some species, heavier in others, second basal with long, feathered seta on outer margin. Fifth feet in male asymmetrical, uniramous, right 4-jointed, left 3-jointed; right resembles forceps, end joint claw- or hook-like; end joint of left not curved to form part of grasping organ.

Acartia clausi Giesbrecht

Figures L and M

Acartia clausi, Giesbrecht, 1892, p. 522; pl. 30, figs. 2, 6, 9, 13-15, 17, 28, 36, 37; pl. 42, fig. 32; pl. 43, figs. 3, 5, 14.
Acartia clausi, Giesbrecht and Schmeil, 1898, p. 152, figs. 150, 151.
Acartia clausi, Sars, 1903, p. 150; pl. 101.
Acartia clausi, Van Breemen, 1908, p. 156, fig. 172.
Acartia (*Acartiura*) *clausi*, Steuer, 1915, p. 394.
Acartia clausi, Willey, 1920, p. 20*k*.
Acartia clausi, Pesta, 1920, p. 546, text-fig. F, 64.

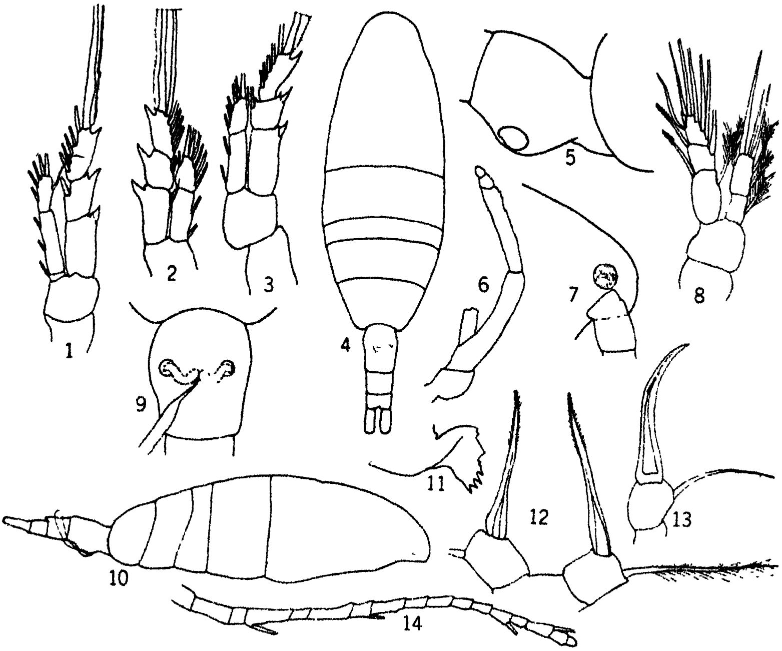

Fig. L. *Acartia clausi*, female. 1: Fourth foot. × 140. 2: Second foot. × 140. 3: Third foot. × 140. 4: Entire animal, dorsal. × 48. 5: Genital segment, margin of thorax, from side. × 140. 6: Posterior antenna. × 140. 7: Head, from side. × 140. 8: First foot. × 140. 9: Genital segment, from below. × 140. 10: Entire animal, from side. × 48. 11: Blade of mandible. × 140. 12: Fifth pair of feet. × 282. 13: Fifth foot, from right side, while attached to body. × 282. 14: Anterior antenna. × 64.

Characters.—Body of female more robust than male (cf. fig. L, 4, 10, and fig. M, 3, 11); as seen from above anterior margin of head of male less rounded than in female; posterior edge of thorax rounded in both sexes (fig. L, 5, 10; fig. M, 11, 12); rostral filaments lacking.

No spines or hairs on margin of last thoracic segment in female; in male from 2 or 3 to 8 or 9 delicate hairs and occasionally a longer and heavier, spine-like hair on dorsal border near posterior edge (fig. M, 12); usually a hair in mid-dorsal line of second thoracic segment at anterior border. Suture line between head and thorax faint, at best, in female and apparently lacking in some specimens. Relative lengths of segments of cephalothorax along mid-dorsal line, female, 52: 17: 7: 13: 15; male, 50: 18: 6: 15: 12.

Abdomen of female 3-segmented, relative lengths of segments and furca, 17: 7: 4: 8; of male 5-segmented, relative lengths with furca 9: 11: 6: 4: 4: 7; first segment of female convex along ventral side, protrusion in middle of length (fig. L, 5, 10); seminal receptacle of characteristic W-shape (fig. L, 9).

Anterior antennae of female when reflexed reach to end of thorax; in male, joint proximal to geniculation of right antenna has row of fine teeth almost entire length; heavy, spur-like spine projects from distal end of next proximal joint, spur one-fifth length of toothed joint (fig. M, 2).

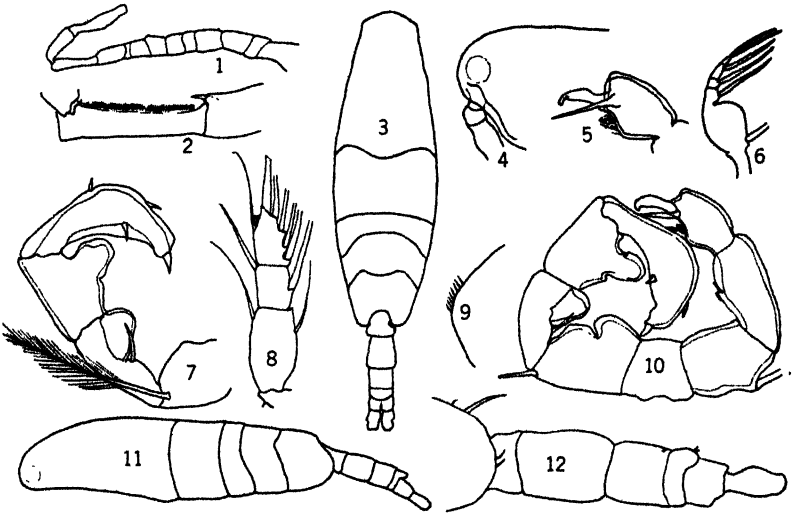

Fig. M. *Acartia clausi*, male. 1: Grasping antenna. × 64. 2: Denticulation on grasping antenna, proximal to geniculation. × 204. 3: Entire animal, dorsal. × 64. 4: Anterior part of head, base of anterior antenna, from side. × 140. 5: Terminal joint of left fifth foot. × 282. 6: Terminal part of posterior maxilliped. × 282. 7: Left fifth foot. × 282. 8: Outer ramus of first foot. × 282. 9: Margin of thorax, from side, to show spines. × 64. 10: Fifth pair of feet. × 282. 11: Entire animal, from side. × 64. 12: Abdomen and posterior margin of thorax, from side. × 140.

Fifth feet of female 3-jointed (fig. L, 2), basal part common to both feet, middle joint quadrangular from in front or behind (fig. L, 12) but not from side (fig. L, 13); terminal part, seen from before or behind, a heavy, straight stylet six times longer than greatest width, row of spines on each edge for distal third of length (fig. L, 12); seen from side stylet has characteristic sickle curve backwards (fig. M, 13). In male, second and third joints of right foot with blunt, lamellar projection on inner margin; end joint with characteristic curve, over three times as long as greatest width, 2 heavy spines on outer margin, 1 at tip, 1 on inner curve but not on margin itself (fig. M, 10). In left foot end joint not half length of same joint in right, nearly as broad as long, on distal margin 1 straight and more slender spine and 1 heavier, crooked, bent sharply at tip to make hook (fig. M, 5, 7).

Dimensions.—Length of female (average), 1.28 mm.; of male (average), 1.26 mm.

Distribution.—Farran (1910, p. 75) gives the distribution as North Atlantic, Mediterranean, Black Sea, off Ceylon, from Spitzbergen to the Azores and Gulf of Guinea. Thompson and Scott (1897, pp. 76, 87) reported it from the Gulf of St. Lawrence and Puget Sound; Williams (1906, p. 648; 1907, p. 72) from Narragansett Bay and Charlestown Pond; Willey (1920, p. 20*k*) found it at several stations of the Canadian Arctic Expedition. The species is very common in the surface hauls at the pier of the Scripps Institution, in winter and early spring (Esterly, 1923).

Remarks.—The copepods here called *A. clausi* very closely resemble those described as this species by others, but there are minor differences. The specimens from the bay lack hairs or denticles on the posterior margin of the thorax and of the abdominal segments. Giesbrecht (1892, p. 513), Sars (1903, p. 150) and Giesbrecht and Schmeil (1898, p. 152) mention teeth or "spitzen" in those locations. The fifth feet of our female specimens are almost identical in structure with those shown by Giesbrecht (1892, pl. 30, fig. 28) and by Sars (1903, pl. 101). There are some differences in proportional lengths of the body segments of our specimens and those described by Giesbrecht and by Sars, but the drawings in Giesbrecht, plate 42, figure 32, and plate 43, figure 5, and in Sars, plate 101, differ from each other as much as the San Francisco specimens do from the figures of either of those authors. It does not seem to me that a new species is required for the animals from the bay, especially since the fifth feet of both sexes have the typical structure of *clausi.*

Acartia tonsa Dana

Figures N and O

Acartia tonsa, Giesbrecht, 1892, p. 522; pl. 30, figs. 7, 24, 34; pl. 43, figs. 6, 10.
Acartia tonsa, Giesbrecht and Schmeil, 1898, p. 154.
Acartia tonsa, Wheeler, 1899, p. 183, fig. 20.
Acartia tonsa, Esterly, 1905, p. 204, fig. 49.
Acartia tonsa, Sharpe, 1910, p. 414, fig. 5.
Acartia (*Acanthacartia*) *tonsa*, Steuer, 1915, p. 396.

Characters.—Two well defined rostral filaments (fig. N, 1, 3; fig. O, 1); form of body similar in sexes but male more slender (cf. fig. N, 10, and fig. O, 3); head and thorax rounded in both sexes as seen from side (fig. N, 1, 4, 5, 11; fig. O, 1, 5, 12, 16).

Posterior margin of thorax with spines or denticles, differing in sexes; in female (fig. N, 4, 5, 6, 11) usually dorsal and ventral groups, spines in latter longer and more slender as rule; in male (fig. O, 5, 6, 7, 12, 13, 16), spines not so numerous, apt to be longer, unusual to find two groups, some specimens with but one spine (fig. O, 5), number and arrangement differing in individuals. Relative lengths of segments of cephalothorax, female, 43: 15: 8: 14: 6; male, 47: 15: 5: 14: 8.

Relative lengths of abdominal segments and furca, female, 11: 4: 2: 5, first segment very convex ventrally; first and second segments with row of small teeth on posterior border, in some animals extending down on sides in first (fig. N, 5), only on dorsal surface in second. Relative lengths of segments and furca in male 22: 32: 18: 6: 9: 15, first segment with tuft of hairs on each side (fig. O, 2, 8), row of delicate hairs each side of last segment; posterior, dorsal margin of second to fourth with row of small teeth which, in second, extend along sides (fig. O, 2, 8).

Anterior antennae extend back as far as middle of genital segment; joint proximal to geniculation in right antenna of male has row of slender spines,

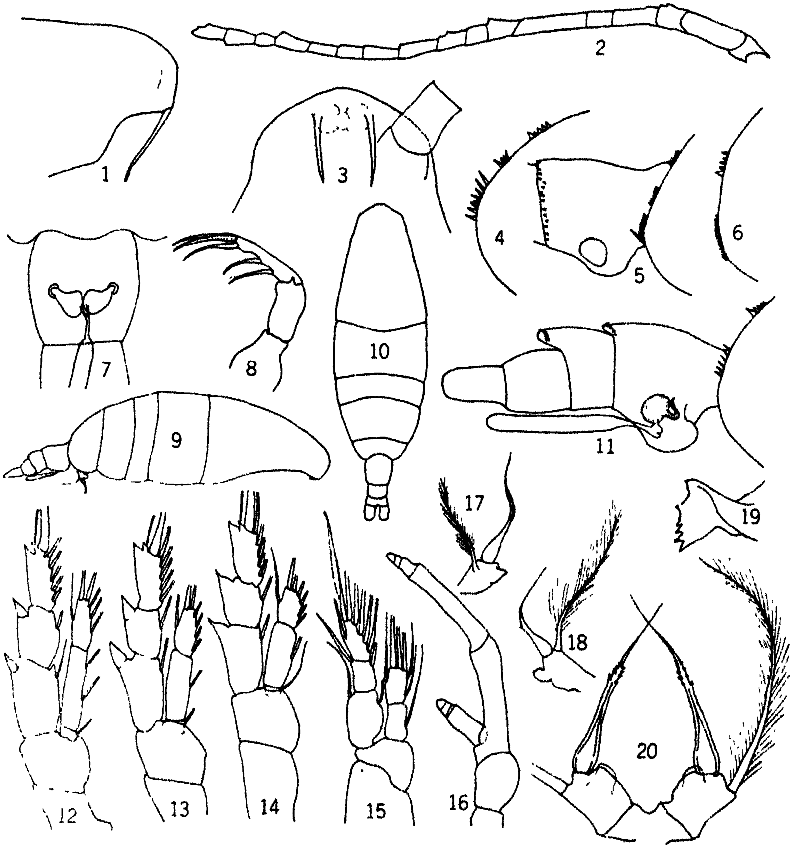

Fig. N. *Acartia tonsa*, female. 1: Head, from side. × 140. 2: Anterior antenna, from ventral side, while attached to body. × 64. 3: Head, from below. × 140. 4: Outline of posterior margin of thorax, to show spines, from side. × 140. 5: Genital segment, margin of thorax, from side. × 140. 6: Outline of posterior margin of thorax, to show spines, from side. × 140. 7: Genital segment, from below. × 140. 8: Terminal part of posterior maxilliped. × 204. 9: Entire animal, from side. × 32. 10. Entire animal, dorsal. × 32. 11: Abdomen and furca, margin of thorax, from side. × 140. 12: Fourth foot. × 140. 13: Third foot. × 140. 14: Second foot. × 140. 15: First foot. × 140. 16: Posterior antenna, from ventral side while attached to body. × 140. 17, 18: Fifth feet of different specimens, from side while attached to body. × 140. 19: Blade of mandible. × 140. 20: Fifth pair of feet. × 204.

and slender, curved spine on next joint proximal extends forward half distance to geniculation (fig. O, 14).

Terminal part of fifth foot of female more slender than in *clausi*, 8 times as long as greatest width and 3 or 4 times length of middle joint (fig. N, 20); end joint serrate on each margin near middle of length. When foot is seen not attached to body, middle joint quadrangular, longer than broad, third joint straight (fig. N, 20); seen from side (as attached to body) (fig. N, 17, 18) middle joint not quadrangular, a conspicuous, tongue-like process at distal, anterior angle; this process looks something like an inner ramus folded over in figure N, 20; in side view, also, end joint curves backward in characteristic way, unlike that in *clausi*.

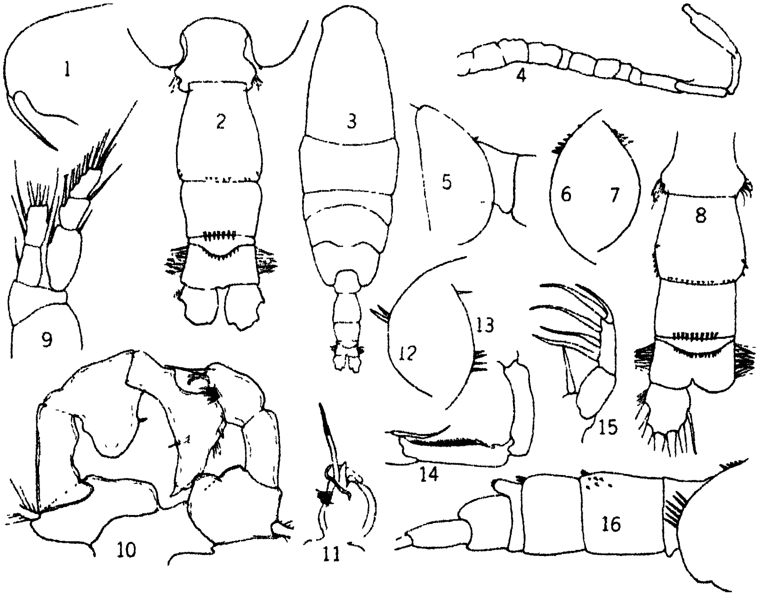

Fig. O. *Acartia tonsa*, male. 1: Head, from side. × 140. 2: Abdomen and furca, dorsal. × 140. 3: Entire animal, dorsal. × 48. 4: Grasping antenna. × 64. 5: Last thoracic and first abdominal segments, from side. × 140. 6, 7: Outlines of margin of last thoracic segment of different individuals, from side, to show spines. × 140. 8: Abdomen and furca, dorsal. × 140; a different specimen than in 2. 9: First foot. × 140. 10: Fifth pair of feet. × 282; right foot at left. 11: End joint of left fifth foot. × 282; from a second specimen. 12, 13: Outlines of margin of last thoracic segment in two individuals, from side, to show spines. × 140. 14: Joints of anterior antenna proximal and distal to geniculation, proximal one at left. × 140. 15: Terminal portion of posterior maxilliped. × 204. 16: Abdomen and margin of last segment of thorax, from side. × 204.

Fifth feet of male (fig. O, 10, 11) characteristically different from *clausi;* second joint without blunt, lamellar process, third joint with it; end joint of left foot varies (cf. fig. O, 10, 11), but always has a straight bristle as long as or longer than the joint.

Dimensions.—Length of female, 1.4 mm.; of male, 1.34 mm.

Distribution.—This species has been reported from Port Jackson, New South Wales, the west coast of South America between Valparaiso and Callao. In American waters it has been found in Jamaica Bay, N. Y., and Plymouth

Harbor (Sharpe, 1910, pp. 407, 414). Sharpe states it occurs abundantly in nearly all the tow examined in the Woods Hole region, and even in the brackish water ponds. Wheeler (1899, p. 183) lists it as one of the commonest copepods at the wharf of the U. S. Fish Commission in July and August, 1899. According to Williams (1906, p. 648) *A. tonsa* is abundant in the summer in Charlestown Pond, where it is the predominant copepod; he also (1907, p. 71) has found it in Narragansett Bay in summer. This species has not been reported from the Pacific Coast except from the San Diego region (Esterly, 1905, p. 204); it is very common in San Diego Bay, and also at the surface at the pier at La Jolla, where it is more abundant during the warmer months (Esterly, 1923).

Remarks.—Our specimens come very close to *A. tonsa* as described by Giesbrecht, especially in the structure of the fifth feet. The females from the bay lack the lateral hairs on the last segment of the abdomen. The process on the middle joint of the fifth foot of the female has not been described, apparently, for other animals, but a similar structure is suggested in Giesbrecht, plate 30, figure 24.

Family Tortanidae

Genus **Tortanus** Giesbrecht

Corynura, Giesbrecht, 1892, pp. 76, 525, 530.
Corynura, Wheeler, 1899, p. 184.
Tortanus, Giesbrecht and Schmeil, 1898, p. 157.
Tortanus, Williams, 1906, p. 648.
Tortanus, Van Breemen, 1908, p. 162.
Tortanus, Scott, 1909, p. 189.

Characters.—Head without lateral hooks, median eye large, without lenses. Cephalothorax symmetrical in both sexes; in some species fourth and fifth segments of thorax fused, so cephalothorax has 5 visible segments, in others fourth and fifth are separate so 6 visible segments. Rostrum absent. A crescentic lamella fringed with fine setae, in front of upper lip. Abdomen of female 2- or 3-segmented, asymmetrical, in some species twisted about longitudinal axis; in male 5-segmented, symmetrical in some species, asymmetrical in others.

Anterior antennae of female symmetrical, 17-jointed; in male, right antenna of grasping type, middle part dilated. Maxilla consists only of first basal and two proximal lobes of inner border, bristles heavy. First maxilliped has proximal lobes entirely aborted, posterior maxilliped 3-jointed.

Inner ramus of first feet 2-jointed in most known species, 3-jointed in at least one; inner ramus of second to fourth pairs 2-jointed; outer ramus of the four pairs 3-jointed. Fifth feet of female uniramous, 2- or 3-jointed, slightly asymmetrical in some species; in male, asymmetrical, each foot 3-jointed, right cheliform, shorter than left.

Tortanus discaudatus Thompson and Scott

Figure P

Corynura discaudata, Thompson and Scott, 1897, p. 80; pl. 6, figs. 1–11; pl. 7, figs. 1, 2.
Tortanus discaudatus, Giesbrecht and Schmeil, 1898, p. 158.
Corynura bumpusii, Wheeler, 1899, p. 184, fig. 21.
Tortanus discaudatus, Van Breemen, 1908, p. 162, fig. 180.

Characters.—Male more slender than female, head rounded in each, fringe of setae in front of mouth (fig. P, 1, 2, 4, 5, 10). Last segment of thorax in female ends on each side in curved, pointed process directed laterally and ventrally (fig. P, 38); last segment rounded in male (fig. P, 2, 9).

Abdomen of female 3-segmented, asymmetrical (fig. P, 3, 8), proportional lengths of segments and furca 15: 7: 10: 25; left blade of furca shorter and narrower than right; in some specimens right blade with broad, flat, pointed process in position of outer marginal bristle as found on left; process may have slender bristle at distal end (fig. P, 6, 7, 8).

Abdomen of male 5-segmented, asymmetrical, bent toward right and slightly twisted (fig. P, 9, 16); first segment prolonged at left, posterior angle, second segment more prolonged at right, posterior angle (fig. P, 9); furca asymmetrical, left blade the shorter and narrower, right blade swollen at level of insertion of outer marginal bristle, which is much heavier than, and twice as long as, the one on the left blade (fig. P, 1, 16).

Anterior antennae of female 17-jointed (fig. P, 1) (Thompson and Scott, 1897, p. 80, say 18-jointed), extend to posterior border of genital segment; right antenna of male geniculated between joints 14 and 15, middle joints swollen (fig. P, 22); the two joints proximal to geniculation with finely pectinate anterior border, basal third of joint distal to geniculation similar (fig. P, 19). Thompson and Scott (1897, pl. 6, fig. 10) show 5 joints beyond geniculation, but in the Woods Hole specimens (Wheeler, 1899, fig. 21 *c*) and in those from San Francisco there are 2.

Specimens from bay agree in main with Wheeler's descriptions of other head appendages (1899, pp. 184, 185), but inner lobe of maxilla has 10 bristles (fig. P, 11); Woods Hole specimens have 11; Thompson and Scott (pl. 6, fig. 6) show 9. Anterior maxillipeds (fig. P, 21) with 7 heavy, curved bristles; posterior maxilliped (fig. P, 12) has 3 heavy, coarsely spinose bristles on first basal (Wheeler states 3; Thompson and Scott, pl. 6, fig. 7, show 4). Inner ramus of first foot 3-jointed, of second to fourth 2-jointed (fig. P, 13, 15, 24).

Fifth feet of female (fig. P, 17, 18) with basal part common to both sides; 2 free joints, distal one without hairs or spines, first with 1 delicate outer marginal; left foot slightly the longer. Right foot of male a massive forceps, middle joint broader than long, prominent outgrowth on inner margin, end joint slender, curved (fig. P, 20).

Dimensions.—Length of female, 1.8 mm.; of male, 1.65 mm.

Distribution.—Puget Sound and Gulf of St. Lawrence (Thompson and Scott, 1897); Woods Hole and Vineyard Sound (Wheeler, 1899, p. 186); various local points in the Woods Hole region in 1908 (Sharpe, 1910, p. 407). Willey (1920, p. 22*k*) found it at three stations, and he states that it "comprises

50–75 per cent of the summer copepod plankton off Souris, Prince Edward Island, and is very abundant off the Biological Station at St. Andrews, N. B.'' This species, or one closely resembling it, occurs in small numbers at the pier of the Biological Station at La Jolla.

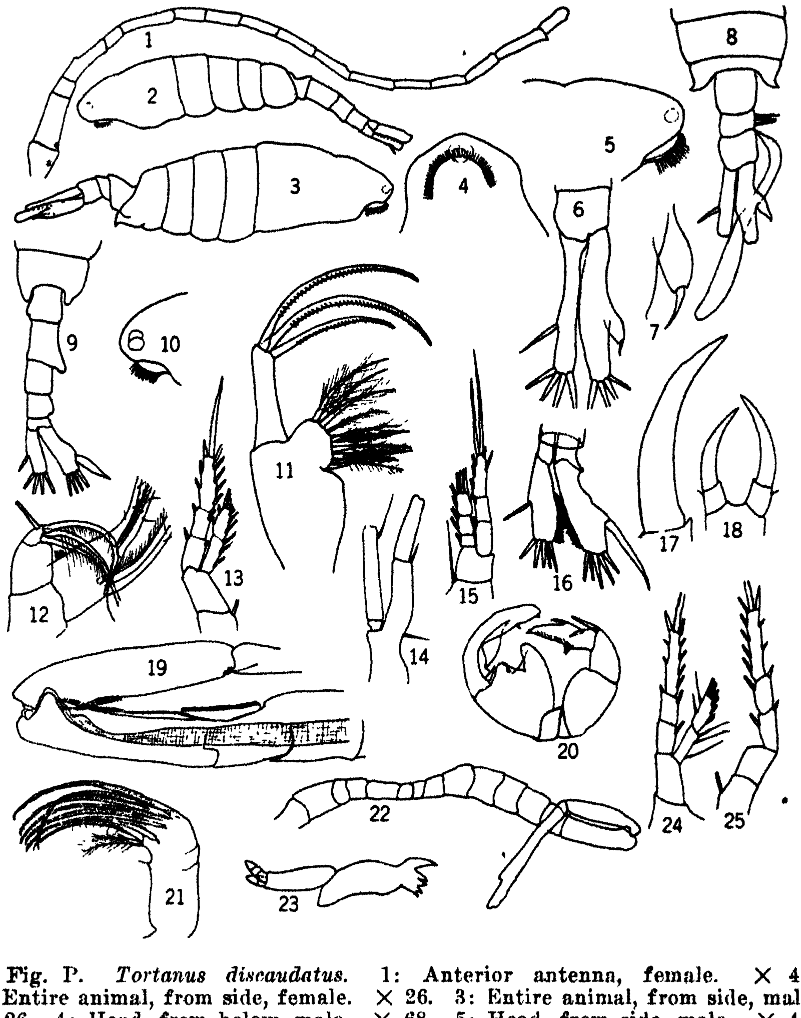

Fig. P. *Tortanus discaudatus.* 1: Anterior antenna, female. × 48. 2: Entire animal, from side, female. × 26. 3: Entire animal, from side, male. × 26. 4: Head, from below, male. × 68. 5: Head, from side, male. × 48. 6: Furca and last segment of abdomen, dorsal, female. × 64. 7: Appendage on right blade of furca, dorsal, female. × 140. 8: Abdomen and last two segments of thorax, dorsal, female. × 34. 9: Abdomen and last thoracic segment, dorsal, male. × 34. 10: Head, from side, female. × 48. 11: Maxilla, male. × 140. 12: Posterior maxilliped, male. × 140. 13: Second foot, female. × 64. 14: Posterior antenna, female. × 64. 15: First foot, female. × 64. 16: Furca, from above, male. × 64. 17: Fifth foot, female. × 140. 18: Fifth pair of feet, female. × 64. 19: Region of geniculation of grasping antenna, male. × 140. 20: Fifth pair of feet, male. × 64. 21: Anterior maxilliped, male. × 64. 22: Right anterior antenna, male. × 48. 23: Mandible, male. × 64. 24: Third foot, male. × 64. 25: Fourth foot, male. × 64.

PART II. THE DISTRIBUTION AND OCCURRENCE OF THE COPEPODA IN SAN FRANCISCO BAY

The following is a list of the stations on the tags in the bottles sent to me; unless otherwise specified the stations are among those designated as hydrographic (H) by Sumner, *et al.* (1914, pp. 150–184).

4986–5005, 5007–5013, 5015, 5016, 5082–5104, 5106, 5107, 5109–5120, 5122–5128, 5130, 5131, 5133–5140, 5143, 5145–5147, 5152–5155, 5158–5160, 5162–5174, 5176, 5179, 5180, 5184, 5190, 5193, 5196, 5199, 5202, 5217–5219, 5228, 5230, 5232, 5242, 5248, 5250–5254, 5257, 5259, 5262, 5266, 5267, 5269–5271, 5275, 5280, 5286–5288, 5291, 5293, 5294, 5298–5301, 5303–5315, 5317–5320, 5325, 5337, 5338, 5340, 5342, 5343, D 5285, D 5311, D 5735, ''Sausalito special.''

NOTE.—At H 5128 twelve hauls were made, according to Sumner (1914, p. 161) and of these I received the specimens collected at 7 A.M., 8 A.M., 9 A.M., 11 A.M., 12 noon, 3 P.M., 4 P.M.

Some of these 182 collections must be omitted when distribution is considered, for the following reasons: Bottle broken, 5015, 5342; dried up, 5184; did not contain copepods, 5136, 5154, 5219, 5250, 5275; not given in Sumner's list, D 5285, D 5311; outside bay, D 5735.

In the following lists there appear the station numbers at which each of the eleven species of copepods were found, together with the numbers of individuals.

Calanus finmarchicus: H 4987, 1; H 4989, 6; H 4993, 80; H 4998, 1; H 4999, 10; H 5002, 4; H 5004, 5; H 5010, 255; H 5082, 5; H 5083, 1; H 5091, 1; H 5093, 1; H 5096, 2; H 5100, 1; H 5102, 1; H 5114, 1; H 5120, 1; H 5123, 1; H 5128, 8 A.M. 1, 9 A.M. 2, 11 A.M. 1, noon 1; H 5130, 1; H 5134, 5; H 5162, 1; H 5252, 7; H 5253, 8; H 5254, 1; H 5267, 1; H 5269, 1; H 5270, 1; H 5298, 7; H 5301, 1; ''Sausalito special,'' 1800.

Paracalanus parvus: H 4998, 1; H 5010, 3; H 5013, 2; H 5106, 1; H 5124, 1; ''Sausalito special,'' 900.

Clausocalanus arcuicornis: H 5110, 1; ''Sausalito special,'' 6.

Ctenocalanus vanus: ''Sausalito special,'' 7.

Eurytemora hirundoides: H 5087, 5; H 5088, 20; H 5101, 1; H 5110, 1.

Metridia sp.: ''Sausalito special,'' 5.

Lucicutia flavicornis: ''Sausalito special,'' 1.

Paralabidocera amphitrites: H 4986, 5; H 4987, 160; H 4988, 8; H 4989, 310; H 4990, 2; H 4991, 10; H 4992, 8; H 4993, 1200; H 4994, 100; H 4995, 8; H 4996, 30; H 4997, 1; H 4998, 20; H 4999, 20; H 5000, 5; H 5001, 1; H 5002, 135; H 5003, 3; H 5004, 15; H 5005, 20; H 5007, 4; H 5008, 6; H 5009, 6; H 5011, 40; H 5012, 12; H 5013, 75; H 5016, 4; H 5082, 90; H 5083, 250; H 5084, 60; H 5085, 1; H 5089, 2; H 5090, 1; H 5091, 210; H 5092, 105; H 5093, 75; H 5094, 53; H 5095, 50; H 5096, 60; H 5097, 7; H 5098, 15; H 5099, 10; H 5101, 40; H 5102, 18; H 5103, 200; H 5104, 1600; H 5106, 225; H 5107, 1000; H 5109, 11; H 5110, 1; H 5111, 7; H 5112, 300; H 5113, 50; H 5114, 200; H 5115, 250; H 5116, 60; H 5117, 95; H 5118, 45; H 5119, 15; H 5120, 17; H 5122, 140; H 5123, 17; H 5124, 30; H 5125, 25; H 5126, 11; H 5127, 50; H 5128, 7A.M. 2, 8 A.M. 9, 9 A.M. 20, 11 A.M. 1, 4 P.M. 2; H 5130, 15; H 5131, 6; H 5133, 1; H 5134, 18; H 5135, 1; H 5137, 25; H 5138, 45; H 5139, 9; H 5140, 4; H 5143, 3; H 5145, 5; H 5146, 10; H 5147, 40; H 5152, 10; H 5155, 1; H 5158, 38; H 5159, 25; H 5160, 20; H 5162, 32; H 5163, 150; H 5164, 300; H 5165, 22; H 5166, 3; H 5167, 17; H 5168, 40; H 5169, 8; H 5170, 5; H 5171, 13; H 5172, 4; H 5173, 1; H 5176, 1; H 5179, 30; H 5180, 1; H 5193, 3; H 5196, 1; H 5199, 70; H 5202, 6; H 5217, 20; H 5218, 1; H 5228, 21; H 5230, 6; H 5232, 4; H 5242, 1; H 5248, 1; H 5251, 4; H 5252, 45; H 5253, 40; H 5254, 30; H 5257, 5; H 5259, 20; H 5262, 7; H 5266, 30; H 5267, 20; H 5269, 40; H 5270, 10; H 5271, 11; H 5280, 2; H 5286, 3; H 5287, 30; H 5288, 37; H 5291, 16; H 5292, 5; H 5293, 5; H 5294, 1; H 5299, 20; H 5300, 5; H 5301, 75; H 5303, 40; H 5304, 160; H 5305, 35; H 5306, 16; H 5307, 7; H 5308, 3; H 5309, 30; H 5310, 3; H 5311, 10; H 5312, 20; H 5313, 750; H 5315, 4; H 5317, 30; H 5318, 11; H 5319, 220; H 5320, 12; H 5325, 2; H 5337, 5; H 5338, 3; H 5340, 1; H 5343, 2; ''Sausalito special,'' 45.

Acartia clausi: H 4986, 1; H 4989, 3; H 5001, 3; H 5002, 5; H 5004, 3; H 5008, 1; H 5010, 1; H 5011, 5; H 5013, 38; H 5016, 2; H 5082, 4; H 5083, 1; H 5086, 1; H 5093, 11; H 5094, 11; H 5096, 35; H 5097, 1; H 5103, 20; H 5106, 7; H 5109, 27; H 5110, 200; H 5111, 4; H 5116, 1; H 5120, 1; H 5122, 1; H 5128, 9 AM. 1, 3 P.M. 1; H 5130, 4; H 5158, 1; H 5160, 1; H 5162, 1; H 5163, 3; H 5170, 1; ''Sausalito special,'' 90.

Acartia tonsa: H 5096, 6; H 5158, 1; H 5160, 4; H 5163, 2; H 5164, 1; H 5170, 3; ''Sausalito special,'' 135.

Tortanus discaudatus: H 5106, 1; H 5122, 1; H 5125, 1; ''Sausalito special.'' 30.

From the lists just given it will be seen that *Calanus finmarchicus* was obtained in 34 hauls at 31 stations; *Paracalanus parvus* in 6 hauls at 6 stations; *Clausocalanus arcuicornis* in 2 hauls at 2 stations; *Ctenocalanus vanus* in 1 haul at 1 station; *Eurytemora hirundoides* in 4 hauls at 4 stations; *Metridia* sp., and *Lucicutia flavicornis* in 1 haul at 1 station; *Paralabidocera amphitrites* in 160 hauls at 156 stations; *Acartia clausi* in 34 hauls at 33 stations; *Acartia tonsa* in 7 hauls at 7 stations; *Tortanus discaudatus* in 4 hauls at 4 stations.

In one haul, designated on the tag in the bottle as ''Sausalito,'' specimens were obtained of each of the eleven species except *Eurytemora,* and it was the only one in which *Ctenocalanus, Metridia,* and *Lucicutia* were found. This collection evidently was made outside the regular series; the duration was not given; the date was February 2, 1912. Judging from the name, Sausalito, the haul must have been located within the middle section of the bay.

There are six other hauls, besides ''Sausalito,'' whose duration is not known or is uncertain: 4992, 5146, 5173, and 5196 belong to the upper division of the bay; 5000 to the middle division; and 5180 to the lower division. In comparing different hauls or groups of hauls, those of unknown duration have been omitted when the number of animals per hour is concerned.

The occurrence of the various copepods that appeared at two or more stations will be taken up first. The entire period of collection will be dealt with as a whole, but the data for the three regions of the bay will be given separately (table 1).

TABLE 1

THE OCCURRENCE OF EIGHT SPECIES OF COPEPODS IN THE THREE DIVISIONS OF THE BAY, THROUGHOUT THE WHOLE PERIOD OF OBSERVATION

Total number of hauls

Lower bay, 70; middle bay, 56; upper bay, 46

	Calanus finmarch.	Paracal. parvus	Clauso. arc.	Eurytem. hirund.	Paralab. amphit.	Acartia clausi	Acartia tonsa	Tort. dis.
A. Number of successful hauls								
Lower	13	2	0	0	68	16	5	0
Middle	16	3	1	1	50	8	1	3
Upper	5	1	1	3	42	10	1	1
B. Total number of hours of hauling								
Lower bay, 11.9; middle bay, 10; upper bay, 6.7								
C. Total number of animals in all hauls								
Lower	288	5	0	0	3904	122	14	0
Middle	1914	902	6	1	4078	119	135	33
Upper	14	1	1	26	2552	249	3	1
D. Number of animals in hauls of known duration								
Lower	288	5	0	0	3903	122	14	0
Middle	114	2	1	1	4028	29	0	3
Upper	14	1	1	26	2532	249	3	1
E. Number of animals per hour (from B and D)								
Lower	24	.42	0	0	328	10	1.1	0
Middle	11	.2	1	1	402	3	0	.3
Upper	2	15	.15	4.	378	37	.45	.15
F. Per cent of successful hauls (frequency)								
Lower	19	3	0	0	97	23	7	0
Middle	29	5	2	2	89	14	2	5
Upper	11	2	2	7	91	22	2	2

The omission of the Sausalito haul from part D of table 1 affects chiefly the relative numbers of *Calanus, Paracalanus, Clausocalanus,* and *Acartia tonsa* in the three divisions of the bay. The abundance of *Eurytemora, Paralabidocera, Acartia clausi,* and *Tortanus* as between the upper, the lower, and the middle bay is not changed by the omission of this particular haul.

Parts C, D, and E of table 1 agree in showing that *Eurytemora* is most abundant in the upper division of the bay; *Paralabidocera* in the middle section; *Acartia clausi* in the lower portion, and *Tortanus* in the middle one. Furthermore, we may safely conclude that *Calanus, Paracalanus, Clausocalanus,* and *Acartia tonsa* are all more abundant in the middle division of the bay than in the others. Since *Ctenocalanus, Metridia,* and *Lucicutia* were found only in the Sausalito haul, they, too, should be looked upon as occurring in largest numbers in the middle part of the bay. While it is true that the Sausalito haul is not comparable with those of known durations, it is not necessary to have a standard measure to see that the largest number of species was obtained in the middle division of the bay. We do not know why other hauls in this division did not obtain as many species as the Sausalito haul did, but it is a significant fact that the only haul that obtained ten species out of eleven was made in the middle section.

It would be well to recognize that *Ctenocalanus, Metridia,* and *Lucicutia* are genera that are typical of the open sea outside the bay. At any rate, they have all been taken in the collections made by the Scripps Institution for Biological Research in the San Diego region, and certain species of *Ctenocalanus* and *Metridia* have been obtained at the pier at La Jolla. *Clausocalanus, Paracalanus,* and *Calanus* occur in very large numbers in the waters around La Jolla, and elsewhere, no doubt, up and down the coast. The two species of *Acartia* are found in swarms at times at the station pier at La Jolla, and *tonsa* is very plentiful in San Diego Bay (Esterly, 1905, p. 205).

Parts D and E of table 1 show that three forms, *Calanus, Paracalanus,* and *Acartia tonsa* were more abundant in the lower division of the bay; in the middle division, *Paralabidocera* was more abundant; *Eurytemora* and *Acartia clausi* were more abundant in the lower division. It is hard to believe, however, that parts D and E show relative abundance more accurately than part C, although the actual figures in part E are all comparable while those in part C are not.

The regular series of collections by the U. S. steamer "Albatross" were made in six different periods of the year (Sumner, 1914, p. 14), approximately two months apart. There are available 163 hauls of known duration and it is possible, therefore, to get some insight into the seasonal occurrence of the Copepoda. The Sausalito haul does not belong to the regular series; *Ctenocalanus, Metridia,* and *Lucicutia* are eliminated from consideration, therefore, while of the other species only *Calanus, Paralabidocera,* and *Acartia clausi* occur often enough and in sufficient numbers to warrant the tabulation of seasonal distribution.

Table 2 gives the abundance and the per cent of successful hauls for the three species, *Calanus*, *Paralabidocera*, and *Acartia clausi*, in relation to the three regions of the bay and to the six regular periods of observation. The number of hauls in any one period and in a given region is rather small, and the time spent in hauling is correspondingly short, but the table brings out the facts as to the occurrence of those copepoda as based on this series. We should recognize that errors become reduced in extent as the number of observations increases,

TABLE 2

THE SEASONAL OCCURRENCE OF THREE SPECIES OF COPEPODS IN THREE DIFFERENT REGIONS OF THE BAY

		Period of observation					
		I	II	III	IV	V	VI
Total number of hauls	Lower	13	16	12	0	12	15
	Middle	7	8	25	5	1	7
	Upper	6	12	8	6	3	7
Total number of hours of hauling	Lower	1.6	2.8	37		2	2.8
	Middle	95	1.6	4.9	1.1	.25	1.4
	Upper	1.	2.2	1.7	1.3	.6	1.7
Total number of animals A. *Calanus finmarchicus*	Lower	264	5	1		19	0
	Middle	91	2	7	0	0	8
	Upper	7	6	0	0	1	0
Number of animals per hour: B. *Calanus finmarchicus* .	Lower	165	1.8	27		9.5	0
	Middle	96	1.2	1.4	0	0	5.7
	Upper	7	2.7	0	0	1.7	0
Total number of animals: C. *Paralabidocera amphitrites*....	Lower	323	1716	653	.	206	1151
	Middle	1379	1690	401	45	2	202
	Upper	493	1645	120	108	57	97
Number of animals per hour: D. *Paralabidocera amphitrites* .	Lower	202	614	1765	. .	103	412
	Middle	1455	1059	82	41	8	145
	Upper	493	750	71	83	95	57
Total number of animals: E. *Acartia clausi*	Lower	58	58	13		0	0
	Middle	0	21	8	0	0	0
	Upper	4	244	1	0	0	0
Number of animals per hour: F. *Acartia clausi*.....	Lower	36	21	2		0	0
	Middle	0	13	1.6	0	0	0
	Upper	4	111	.6	0	0	0
Per cent of successful hauls (frequency):							
G. *Calanus finmarchicus*	Lower	31	25	8	.	50	0
	Middle	38	25	24	0	0	29
	Upper	29	17	0	0	25	0
H. *Paralabidocera amphitrites* ..	Lower	92	100	100	.....	83	89
	Middle	100	75	80	80	100	86
	Upper	100	75	100	100	100	100
I. *Acartia clausi*.....	Lower	61	25	25	....	0	0
	Middle	0	25	20	0	0	0
	Upper	29	58	11	0	0	0

and there may be some reason for a cautious statement of the meaning of the results in table 2. But there is some value in bringing out the facts as shown by the collections of the "Albatross," even though it is realized that a larger number of hauls might alter some conclusions. As regards the use of the term 'frequency' (the per cent of successful hauls), it should be explained that a haul is considered successful even if it contains only one animal. Frequency is a less accurate index of distribution than is "number of animals per hour," but the two indices taken together are often more significant than either one alone. Frequency shows how generally distributed a form may be; or in how many hauls it might be expected that at least one specimen will be obtained. It is often found that frequency and abundance are not parallel, and in cases of disagreement between them reliance should be placed upon abundance as revealing more accurately the distribution. Michael (1911, p. 116) has discussed the reasons for the lack of agreement between frequency and abundance.

From the facts brought out in parts A and B of table 2 we may safely conclude that *Calanus* is most abundant in the first period of collecting (February 13 to 27, 1912), and in the lower and middle sections of the bay, the former excelling the latter in this respect by a considerable margin. The species is widely scattered in the upper section of the bay, and this is true, also, throughout the rest of the year. It is doubtful if any of the differences shown in part B of table 2 are significant except those between the values for Period I and the other periods and those between the values for the three regions of the bay during the first period. It would be well to note, however, that *Calanus* was not obtained in the eleven hauls made during the fourth period (October 7 to 12, 1912). The frequencies are larger on the whole during the first period (part G of table 2), which is another indication that the species really is more abundant at that time.

The figures showing occurrence and distribution of *Paralabidocera* (C and D, table 2) are sharply contrasted with those for *Calanus*. The former is much more abundant and more generally distributed. It was taken in every period, in each of the regions of the bay, and, more often than not, specimens were found in all the hauls of a given group (part H).

Part D of table 2 shows that *Paralabidocera* is present in larger numbers during the first two periods (February 13 to 27 and April 23 to May 6, 1912) than during any other period. During that time of the year the largest numbers were obtained in the middle region of the bay, with the upper region standing second. As between Periods II and III, there is a marked falling off in abundance in the middle and lower regions and the decline continues in the middle region through period V (November 25 to December 5). It is shown, furthermore, that the region of greater abundance shifts from the middle to the lower part of the bay beginning with Period III (July 22 to 31, 1912). There can be little doubt of the significance of the differences between Periods I and II on the one hand and the rest of the periods on the other. We may conclude, also, that during the periods of greatest abundance, the middle region of the bay is more populous than either of the others.

Acartia clausi is much less abundant than *Paralabidocera,* and somewhat less so than *Calanus.* As shown in part F of table 2, the first three periods are sharply set off from the second three, for not a single specimen was found in hauls taken in Periods IV, V, or VI (October 7 to 12, 1912; November 25 to December 5, 1912; January 13 to 28, 1913). Most of the animals were obtained in the first and second periods, and the middle region of the bay stands in third place, so far as abundance is concerned, during both periods. If Periods I and II are considered together, *Acartia clausi* is more abundant in the upper part of the bay.

In regard to the seasonal occurrence of *Acartia clausi* in other waters, I have shown that for two successive years it was most abundant in March at the pier of the Biological Station at La Jolla, California (Esterly, 1923, p. 425). During July, August, and September the animals were absent from the collections or were found in small numbers. Farran (1910, p. 77) states that this copepod is most abundant in enclosed waters in the warmer parts, of the year; he states, further (1908, p. 87), that the species is "apparently always present at all depths from surface to 1000 fathoms, usually in large numbers."

The other copepods found in the regular series of collections were *Paracalanus, Clausocalanus, Eurytemora, Acartia tonsa,* and *Tortanus.* Only eight specimens of *Paracalanus* were obtained; of these, one was taken in the upper end of the bay during the second period; five in the lower division in the first period; two were found in the middle section of the bay, one of them during the first period and one during the third. These numbers are so small that they do not have much significance. The Sausalito haul, however, was made only a few days before the beginning of Period I, and the haul obtained 900 specimens. The indications are, therefore, that *Paracalanus* is most abundant early in the year.

Clausocalanus was found in one haul of the regular series; there was but one specimen, and the haul was made in the second period, in the upper division.

Eurytemora was found in four hauls of the regular series. The total number of animals obtained was 27; 26 of them were found in hauls made in the second period in the upper division of the bay, while the remaining animal was taken in the middle division during the same period.

Acartia tonsa was found in six hauls in which 17 animals were obtained. Five of these hauls were located in the lower part of the bay and yielded 14 animals; four of the five were made in the third period and obtained 8 animals, while the fifth haul was made during the second period and obtained 6 animals. Three animals were obtained in the remaining haul of the six, which was located in the upper part of the bay during the third period. *Tonsa* appeared, therefore, only in April and July; it was most abundant in the lower part of the bay, where 2.5 animals per hour were taken compared with the 1.7 animals per hour in the upper part; none was obtained in the middle part of the bay during the regular collecting, but we should not forget that the Sausalito haul (middle section, at about Period I) obtained eight times as many animals as all the other successful hauls together.

At La Jolla, *Acartia tonsa* was most abundant during August of the first of two successive years, and in July of the second year, while the smallest numbers were obtained in January and February of the first year and in March and May of the second (Esterly, 1923).

Tortanus was found in the regular hauls only in Period II; three animals were obtained, two in the middle region and one in the upper. The Sausalito haul alone took ten times as many as the others.

The seasonal occurrence of the copepoda obtained in the regular collections may be summarized by listing the species in relation to *period* and *location* in the bay, with reference to the largest numbers of animals per hour, as follows:

Period I. Lower bay, two species at their greatest abundance, *Calanus finmarchicus, Paracalanus parvus.*
Middle bay, one species at its greatest abundance, *Paralabidocera amphitrites.*
Upper bay, no species at greatest abundance.

Period II. **Lower bay, none.**
Middle bay, *Tortanus discaudatus.*
Upper bay, three species reached their greatest numbers, *Acartia clausi, Clausocalanus arcuicornis, Eurytemora hirundoides.*
Period III. **Lower bay, *Acartia tonsa* at its maximum.**

A list, similar to the preceding but dealing with the second largest numbers per hour, is as follows:

Period I. Lower bay, *Acartia clausi.*
Middle bay, *Calanus finmarchicus.*
Upper bay, none.
Period II. Lower bay, *Acartia tonsa.*
Middle bay, *Paralabidocera amphitrites, Paracalanus parvus, Eurytemora hirundoides.*
Upper bay, *Tortanus discaudatus.*

These lists show that in the lower bay, during Period I, three species reached their greatest or second greatest numbers per hour; in the middle portion, two species reached their greatest or second greatest numbers, while in the upper part of the bay no species was found in either largest or second largest numbers per hour. During Period II, in the lower division of the bay, one species reached only its second largest abundance; in the middle section four were found in largest or second largest numbers per hour; in the upper section four species were present in largest or second largest numbers. During Period III, in the lower part of the bay, one species only was found in its largest numbers. All other periods and locations failed to give either largest or second largest numbers per hour for any of the species.

We may state the following conclusions at this point:

1. The best chance of obtaining larger numbers of the species represented in the regular collections would be in the first or second periods; perhaps this entire period would include February, March, and April.

2. As to location, the 'best' regions would be in the lower or middle parts of the bay, on the whole, though for *Eurytemora* the upper part is more favorable.

Temperature and salinity are two measurable hydrographic factors that vary with season and with locality within the bay, as has been shown by Sumner, *et al.* (1914, pp. 28–88). The distribution of bay temperatures is more variable from period to period than is the distribution of salinities. Figure B, page 39, of Sumner's report shows greater differences between the curves than does figure M, page

68. In the latter figure the curves have the same general character; the lowest salinity is at the extreme upper end of the bay, with the highest in the region of the Golden Gate, while the salinity at the lower end of the bay is slightly less than opposite the Gate, but much higher than at the opposite end of the bay. This is the general distribution of the salinities regardless of season, and the differences would probably have some biological significance were salinity the only factor. But there is always an association of temperature and salinity; the water may be warmer or colder, or of higher or lower salinity, but the two factors cannot be separated under natural conditions.

During the two periods when the copepods are most abundant there is a relatively slight range of temperature. This makes it possible to study the effect of variations in salinity when the temperature is practically constant. As is shown in figure B (page 39) of Sumner's report, the total range of temperature during Period I was less than 1° C. It is doubtful whether such a small difference would have any effect on the responses of the organisms. During Period II the temperatures of the water at the ends of the bay are about 2° C. higher than at the center, and the mean temperature (12°99 C.) is less than a degree higher than the mean of the first period, which is 12°22 C.

I have assumed that the temperature differences in the bay during Periods I and II may be neglected and that if salinity affects the distribution of the copepods it will be revealed when the temperature is practically constant and the salinity varies. Furthermore, the primary hydrographic stations have been arranged in six groups, designated as A, B, C, D, E, and F, and the results of the hauls located at these primary stations have been tabulated. The position of the groups of primary stations may be seen in plate 3 of Sumner's report. Group A is composed of stations 5001, 5002, 5003, 5004, 5005; group B of 5006. 5007, 5008; group C of 4967, 4968, 4969, 4970; group D of 4971, 4972, 4973, 4974; group E of 4979, 4980, 4981; group F of 4975, 4976, 4977, 4978. In Sumner's figure M, page 68, it can be seen that on the whole the salinity increases from the region covered by F to the maximum at C and then falls slightly through B and A. The distribution of the temperatures with reference to the six regions may be seen in figure B, page 39. Table 3 gives the results of the hauls at each of the six localities, for the three most abundant copepods.

Calanus was most abundant at localities B and D, and the numbers at the other locations were very much lower. A, B, and C have nearly the same mean salinities for the two periods, yet the numbers per hour taken at B are from thirteen to eighteen times larger than those at A and C. The inference is that some other factor than salinity enters in. The two regions with the lowest mean salinities (E and F) show the lowest numbers per hour of all the locations. The results on the whole are somewhat confusing with the exception of the fact that the abundance is low at localities with low salinity. It is doubtful if the slight differences in temperature that exist between the different localities have any determining effect on the distribution of the species. *Calanus* was most abundant at the very season when the temperature range is least (table 2); the general low temperature may affect abundance, but range of temperature apparently does not.

TABLE 3

THE DISTRIBUTION OF THREE SPECIES OF COPEPODS DURING FEBRUARY 13 TO 27, 1912, AND APRIL 23 TO MAY 6, 1912, IN SIX LOCALITIES IN THE BAY

The results obtained in the two periods are combined. The period is characterized by nearly uniform temperatures and by a range of salinities.

NOTE.—The average temperatures for the two periods were obtained from tables 3 and 4 of Sumner's report; the average salinities from tables 12 and 13.

	Locality					
	A	B	C	D	E	F
Total number of hauls	13	16	8	8	8	11
Total number of hours	1.9	2.4	1.5	1.26	1.4	1.7
Total number of animals:						
Calanus finmarchicus	12	257	12	81	6	7
Paralab. amphitrites	848	1046	78	3196	1645	403
Acartia clausi	117	8	1	70	12	236
Number of animals per hour:						
Calanus finmarchicus	6	107	8	64	4	4
Paralab. amphitrites	445	435	52	2520	1175	234
Acartia clausi	61	3	0.6	55	8	139
Frequency:						
Calanus finmarchicus	31	19	38	25	25	18
Paralab. amphitrites	100	94	88	100	100	72
Acartia clausi	62	44	12	12	38	55
Average temperature	12.94	12.31	12.39	12.67	12.58	12.95
Average salinity	27.56	27.39	28.29	24.44	20.98	14.31

In a study of the data obtained by the Scripps Institution while collecting in the San Diego region, I found that there was little if any consistent relation between surface occurrence of *Calanus* and temperature (Esterly, 1912, pp. 288–292). The period considered included June, July, August, and September. While the numbers of animals obtained in surface hauls vary greatly with the time of day, these copepods were most abundant at temperatures ranging from 18°1 C. to 21°5 C. and least abundant from 16° C. to 18° C. The animals occurred in fewer numbers betwen 8 A.M. and 4 P.M. than at any other time of the twenty-four hours, but if all the surface hauls are considered, regardless of time of day, *Calanus* was much more abundant at temperatures ranging from 18°1 C. to 21°5 C. In spite of this showing, it was concluded (Esterly, 1912, p. 292) that the occurrence of *Calanus* at the surface is not correlated with temperature. Farran (1910, pp. 87–88) states that *Calanus finmarchicus* is eurythermal. It is found in abundance in the English Channel during the summer where the mean surface temperature is 16° C.; also to the north in waters of 0° C. or less. And Farran states further, that, according to Cleve, this copepod has a temperature range in the North Atlantic from 22° C. to 0°2 C.

With respect to the surface occurrence of *Calanus finmarchicus* in the San Diego region in relation to salinity, it was found that, for all surface hauls (apart from time of day), the species was most abundant at a salinity range of 33.605 to 33.648. The animals were most numerous at the surface between 4 P.M. and 4 A.M., and during that time there was no consistent relationship between salinity and abundance. Farran (1910, p. 88) states that *Calanus* is abundant in the North Atlantic only in waters of a salinity of 35.30 or less.

No study of the seasonal occurrence of *Calanus* in the San Diego region has been made, since the available hauls were all made in the summer months. But according to Farran (1910, p. 88), the maximum abundance in the North Atlantic occurs in April or May.

In table 3 it is shown that *Paralabidocera* was overwhelmingly more abundant in locality D, with E coming next. The numbers per hour were relatively low at the other locations; the frequency was high everywhere. The animals were found in fewest numbers at location C with the highest salinity, while locality F, with the lowest salinity, ranks next. Possibly these facts may be interpreted as showing that *Paralabidocera* will be found regularly in larger numbers in regions of moderate salinity than where the salinity is higher or lower. I think it is significant that in locations A, B, and C, where the salinity was high, there were so few animals as compared with the three locations where the salinities were lower. It would be hard to say whether a salinity around 20.00 to 24.00 (as at D and E) represents the optimum for this species, but the question could be answered, no doubt, by observation of a larger number of hauls.

Acartia clausi was so much more abundant at the opposite ends of the bay than elsewhere, that little more need be said. It is hard to suggest a reason for the greater abundance in the region of the lowest salinity when the next greatest numbers per hour were obtained in the locality where the salinity, on the average, was next to the highest. We should note, also, that less than one animal per hour was obtained at C, where the salinity was highest. According to Farran (1908, p. 77), *A. clausi* is absent from regions of low salinity, such as the Baltic Sea. He gives as the widest extremes of salinity: Belt Sea, 18.42 and 14.47; mouth of English Channel, 30.20 and 11.25, as averages. This copepod is mentioned by Farran (1920, p. 20) as a typical example of a euryhaline species.

It is interesting, in this consideration of distribution with respect to salinity, to note that *Eurytemora* was most abundant in the haul made where the salinity was the lowest encountered in the bay. According to Sumner (1914, p. 59) haul H 5088 gave a salinity of only 3.25. In this haul there were found 20 of the 27 specimens of *Eurytemora*. Haul 5087, with a salinity of 3.75, contained 5 specimens.

We have considered in table 3 the distribution of three species obtained in largest numbers during periods of slight variation in temperatures but considerable and characteristic variations in salinity. If it were possible to deal with reasonably large numbers of animals at a period when the salinity is uniform and the temperature varied, we could check the previous observations; but this is not possible because there is always a salinity gradient. Furthermore, most species are obtained in such small numbers that it would be useless to base conclusions on them. During the third period of regular collecting, however, *Paralabidocera* was obtained in fairly large numbers, and it has seemed worth while to show how the animals were distributed. This third period had the greatest temperature range of the entire six, and there was a salinity gradient similar in a general way to those of all the periods.

In showing the distribution of *Paralabidocera* during the third period, I have used the same localities based on primary hydrographic stations that were used in the preparation of table 3.

A comparison of table 4 with table 3 shows that the region of greatest abundance had changed. In July, more animals were obtained in localities A, B, and C; and, of these three, rather more in B and C. It will be seen that the temperatures were lowest at B and C, but it is hard to believe that, as between B and E, a difference in temperature of 0°.55 C. and of 1.30 in salinity will account for the difference in numbers. Here again laboratory experiments would undoubtedly help to explain the apparent inconsistency if the distribution is dependent on salinity or temperature or on both.

DISCUSSION

The foregoing presentation of the data has shown that each of the different species was more abundant in some regions of the bay and less abundant in others. There is no evidence that these plankton animals were uniformly distributed through the bay. There was also a marked seasonal variation in abundance, and so far as this set of observations goes there can be no doubt that the first two periods of regular collecting (February 13 to 27, 1912, and April 23 to May 6, 1912) were the times when the Copepoda were most abundant. The details of the geographical and seasonal distribution have been given in the tables.

TABLE 4

THE DISTRIBUTION OF PARALABIDOCERA DURING JULY 22 TO 31, 1912, IN SIX LOCALITIES IN THE BAY

NOTE.—The average temperatures for the two periods were obtained from table 5 of Sumner's report, and the average salinities from table 14.

	Locality					
	A	B	C	D	E	F
Total number of hauls	7	6	6	10	5	4
Total number of hours	1.2	1.4	1.6	1.9	1.1	.92
Total number of animals	123	208	196	171	72	68
Number of animals per hour	102	149	123	90	65	74
Frequency	86	100	100	90	100	100
Average salinity	29.14	27.93	30.28	27.04	26.63	17.23
Average temperature	18.35	16.48	15.25	16.91	17.03	18.61

When we try to ascertain from the available data whether there were certain physical conditions related to the times or the places of greater abundance, the matter becomes more difficult and uncertain. The relations between abundance of different forms and the temperature and salinity of the water have been pointed out in connection with the tables. Statistical treatment of the observed values might be useful, though the number of hauls is small. But, if it is proper to make suggestions that may prove useful in the future, what is especially needed is an increased number of observations in the field, and also observations in the laboratory on the reactions of the different species.

When the attempt is made to ascertain why a given species of animal is more abundant at one region or time than another, attention naturally turns first to the more easily measured factors, such as the salinity and temperature of the water. These undoubtedly play an important part in the distribution of the plankton copepods. But other factors must be considered. The amount and kind of plankton at a given time and in a given area may be affected by such conditions as currents, winds, the state of the tide, the time of day, and chemical composition of the water. In addition to physical and chemical conditions, there are important biological factors. The latter involve the times of reproduction of the various copepods, their growth, their life histories, their relations to their food organisms, and the relations of these in turn to the whole series of conditions that affect the plankton in general. All such factors and conditions are more or less interlocked in their effects.

If the foregoing is a fair statement, it must be plain that success in the study of the distribution of plankton depends to a considerable degree on having a sufficiently large number of hauls. We should be able to know whether the results in any given year are exceptional. The work of Herdman and his associates in the study of the plankton of the Irish Sea, extending over fifteen years, has shown that one year is, broadly speaking, like another (Herdman, 1922). When, where, and how the collections should be made are matters that can be decided for each given problem, but a series of collections consistently carried out is more important than anything else in the study of plankton production. Kofoid pointed this out in his report on the Illinois River. He says (1903, p. 511),

> If this cyclic movement in plankton production be characteristic of the plankton generally, fresh water and even marine, it must follow that scattered and irregular collections, or those at intervals exceeding a week or at most a fortnight, may fail entirely to give an adequate representation of the course of production or relative fertility of a body of water. Chronological series throughout the whole seasonal range of climatic conditions and at close intervals—of one week or less—are necessary for any accurate delineation of production and fertility of water by the plankton method.

This discussion of the distribution of the copepods of the bay should be looked upon, I think, as an attempt to bring out the facts shown by the available collections. It does not seem advisable to make any general conclusions from the facts presented here, for their chief value will probably be as a source of comparison with the results of future series of collections.

SUMMARY

1. Eleven species of copepods were found in the "Albatross" collections in San Francisco Bay. In one haul ("Sausalito"), not in the regular series, all but one of the eleven species were obtained, and that haul was the only one in which representatives of three of the genera (*Ctenocalanus, Metridia, Lucicutia*) were found. *Paralabidocera amphitrites* was the most abundant species; it was found at all seasons and in all localities and in 161 out of 171 hauls. At the other extreme was the genus *Lucicutia*, of which only one specimen was found.

2. The results of the regular series of collections lead to the conclusion that the copepoda are more abundant in the lower and middle regions of the bay. But if the single "Sausalito" haul is included, as giving an indication of what might be expected as a rule, the copepods are to be found in much larger numbers in the middle region than in the upper or lower divisions of the bay.

3. The seasonal variation in abundance is very marked. The results of the regular series show that the copepoda were much more abundant in the first and second periods of collecting (February 13 to 27 and April 23 to May 6).

4. The distribution with regard to salinity could not be studied to advantage except with three species, on account of the small numbers of animals obtained. But *Calanus finmarchicus*, *Paralabidocera amphitrites*, and *Acartia clausi* were sufficiently abundant in February and April to afford data worth examination. The results for *Calanus* are not clear except that it occurred in smallest numbers at the two stations where the salinity was lowest. It is apparent that *Paralabidocera* was to be found in larger numbers where the salinity ranged from 21 to 24. It was least abundant where the salinity was highest; the next highest abundance was found in the locality where the salinity was lowest. There is nothing in the results to show that the distribution of *Acartia clausi* was affected by salinity. Although it was most abundant in the part of the bay where the salinity was lowest, the region of next abundance was where the salinity was next to the highest point. *Eurytemora* was found only in small numbers, but most of the specimens obtained came from water of a salinity of only 3.25, the lowest encountered in the survey.

5. *Paralabidocera* alone, of all the copepods, was found in large enough numbers in July (Period III) to warrant a study of the occurrence. It is during this period that there is the greatest temperature range in the bay, while there is the same sort of salinity gradient that is found at the other periods. During the third period the difference in salinity between the regions where *Paralabidocera* was most and least abundant was 1.30, and the temperature difference was 0°55 C. It seems doubtful that such slight differences can be of biological significance.

6. As might have been expected, the different species were not found in their greatest abundance at the same season or in the same locality.

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WOOD-BORING MOLLUSKS FROM THE HAWAIIAN, SAMOAN, AND PHILIPPINE ISLANDS

BY

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In the autumn of 1922, under the auspices of the Committee on Marine Piling Investigations of the National Research Council, test boards for marine borers were placed at several localities in the Hawaiian Islands, and at Tutuila, Samoa. In April, 1923, a similar board was placed at Cavite, Manila Bay, Philippine Islands. These test boards, which were of a standard design (Clapp, 1923), were installed and maintained through the coöperation of the Bureau of Yards and Docks of the United States Navy, and the United States Lighthouse Service. At intervals of two weeks a block was removed from each board and forwarded to the Zoological Laboratory of the University of California. In this laboratory the blocks have been examined by the writer, with the assistance of Mr. Harold Kirby, the work being carried on under the direction of Dr. C. A. Kofoid as a phase of the investigations of the San Francisco Bay Marine Piling Committee.

Extremely little is known regarding the marine borers of these islands. The results here reported are not looked upon as affording at all a complete list of the species occurring in these regions, but they do give an idea of the distribution and relative abundance of the commoner and hence more destructive forms.

The molluscan borers have been represented in the test blocks by one species of *Martesia* and seven species of *Teredo,* of which four appear to be distinct from any as yet described. The genus ***Bankia*** has not been represented in any of the material that has come to hand.

The stations from which blocks have been received are: Pearl Harbor (two stations, United States Navy Coaling Plant and Kuahua Island); Honolulu Harbor; Nawiliwili Bay, Island of Kauai; Tutuila, Samoa (United States Naval Station); and Cavite, Philippine Islands.

Genus **Martesia**

Martesia striata Linn.

Plate 8, figures 1–5

This species has occurred occasionally in the blocks from Pearl Harbor, rarely more than three or four small specimens of a maximum length of 2 cm. occurring in any one block. That it is of economic importance in this locality has been shown in an earlier paper (Kofoid and Miller, 1923), where instances were cited and figured of damage to piling and of penetration through redwood sheathing and an inside layer of tarred ship's felt.

Burrows attributed to this species were also found in one of the blocks from Nawiliwili, although the animals themselves had been lost out before the block was sent in, thus precluding certain identification.

At Cavite, attack by this species on the test blocks has been rapid and extremely destructive, the damage by *Teredo* at this locality, although considerable, being secondary to that occasioned by *Martesia*. Blocks exposed four months or longer were usually in a crumbling condition, the surface being riddled by small *Martesia*, mostly less than 2 cm. in length. The deeper portions of the block were at the same time penetrated by the longer burrows of *Teredo*.

Genus **Teredo**

Teredo parksi Bartsch

Plate 9, figures 6–15

This species was described by Bartsch (1921) from piling in Pearl Harbor. The shell is characterized by a broad anterior lobe and a consistently small auricle, characters which appear to exhibit less variability in this species than in most others. The pallets are readily distinguished by the long stalk and short, deeply excavated blade which is covered the greater part of its length by a dark epidermis. The blade is usually more deeply excavated on the outer than on the inner face, a character which is most accentuated in the Samoan specimens. The latter tend also to have more regularly shaped, straight-sided pallets. These characters, however, appear too variable to be of systematic importance. It is possible that further study may indicate a separate category for the Samoan specimens, but the material now at hand does not warrant such a step.

Teredo parksi appears to be the dominant species in the islands, being present in the test blocks from all localities except Nawiliwili. The heaviest attack by this species occurred in Pearl Harbor. Blocks submerged here September 1 showed considerable surface attack at the end of a month, and by the end of the second month a length of 8 cm. had been attained by the largest specimens. At the end of five months the blocks were thoroughly honeycombed and beginning to crumble as a result of the combined attack of *Teredo* and *Limnoria.* The rate of growth under normal conditions at this locality appears to be from 3 to 5 cm. a month during the first five months, at the end of which time the blocks were usually so crowded as to hinder or stop further growth. A burrow 18.5 cm. in length occurred in a block submerged five months at the United States Navy Coaling Plant, and a burrow 22.5 cm. long was found in a block submerged six months at Kuahua Island, these being the maximum lengths recorded.

This species is incubatory, and in Samoan waters becomes sexually mature within a few weeks after entering the wood. Specimens containing well developed larvae in the gills were found in blocks submerged only two months at Tutuila. Potts (1923) reports that rafts in the water only twenty-four days in Pago Pago Harbor contained shipworms which were producing free-swimming larvae; it is probable, although not certain, that he was dealing with this species. In Pearl Harbor *Teredo parksi* appears to mature more slowly and to reach a larger size, the difference doubtless being due to temperature conditions.

In Samoa *Teredo parksi* appears to breed uninterruptedly throughout the year, but in Hawaii the breeding activity, as indicated by settlement of larvae, reaches a maximum in August, September, and October, progressively decreases from November to March, and reaches a minimum, possibly ceasing altogether, in April.

Teredo bartschi Clapp

This species was described by Clapp (1923) from Port Tampa, Florida. Specimens of a *Teredo* prevalent in the later blocks from Nawiliwili compare closely with paratypes of this species which I have received from Mr. Clapp. This species occurs numerously in the blocks placed after September, and sporadically in the earlier blocks.

Teredo diegensis Bartsch

This species, described by Bartsch (1916) from San Diego, occurs sporadically in the blocks from Pearl Harbor, Honolulu Harbor, and Nawiliwili. The specimens from the Islands differ from typical *T. diegensis* in having the ridges on the shell more close-set and the epidermis of the distal portion of the blade of the pallets more transparent, so that the outline of the oval calcareous portion within can be plainly seen. In preserved specimens that we have received from Nawiliwili the distal epidermis is of amber clearness; but the dried pallets have the epidermis darker and more opaque, so that they are not distinguishable from those of specimens from the California coast.

Teredo affinis Deshayes

Plate 11, figures 26–30

Shell similar to that of *Teredo furcillatus,* but with the anterior lobe in general narrower and the auricle broader, differences which have been fairly constant in all the specimens examined.

Pallets with a long, slender stalk; blade consisting of a short, urn-shaped, calcareous base, surmounted by a dark brown, chitinous distal portion, wholly uncalcified, and of very irregular shape. In specimens considered typical the distal portion consists of a narrow, elongate, cupped median extension, very deeply excavated on the outer, less deeply on the inner face; and further cut away on each side nearly to its juncture with the calcareous base, where the chitinous portion is spread out abruptly and slightly excavated to form two shallow lateral cups. As a result of wear the median extension may be excavated nearly to the base within as well as without, so that the pallet appears to end in two slender leathery fingers. The lateral excavations are usually unequal, and one or both may be entirely lacking. The calcareous base is cut off abruptly at its juncture with the distal chitinous portion.

This species was described by Deshayes (1863) from Reunion Isle.

Notwithstanding the irregularity in the form of the pallets, the characteristic leathery distal projections enable them to be recognized almost at a glance. But the chitinous portion rather readily comes free of the calcareous base, which then has a deceptive appearance of completeness, and might be mistaken for a pallet of some other species.

The test board at Nawiliwili was placed February 1, 1923. In the earlier blocks *Teredo affinis* predominates, and is very destructive; but in the blocks placed after September it is almost entirely replaced by *Teredo bartschi* and *Teredo diegensis.* A few specimens of *T. affinis* have been found in the blocks from Honolulu Harbor.

Teredo furcillatus sp. nov.

Plate 10, figures 16–20

Shell with the anterior lobe shorter and narrower than in *Teredo parksi,* and the auricle decidedly longer and broader. The shell is less highly polished and transparent, and the ridges of the anterior median area are more coarsely denticulated than in *T. parksi.*

Pallets with the stem long and the blade small, variable in shape, the distal portion deeply excavated on the outer and usually also on the inner face, although the latter in some cases is only slightly notched. The most distinctive feature of the pallets is the absence of a dark periostracum, the distal portion of the blade being either light yellowish or perfectly white. The name is suggested by the resemblance of the pallets to a small two-tined fork (Latin *furcilla*).

The measurements of the type are: *shell,* height, 3.3 mm.; length, 3.4 mm.; *pallets,* length of blade, 1.7 mm.; width of blade, 1 mm.; length of stalk, 2 mm. The type, from Tutuila, Samoa, has been placed in the Museum of the California Academy of Sciences, San Francisco, as no. 1729. Paratypes have been placed in the collections of the Department of Zoology, University of California, the Academy of Natural Sciences, Philadelphia, and the United States National Museum.

Teredo furcillatus has occurred in limited numbers in the blocks from Tutuila and Honolulu Harbor. The longest burrow recorded was 6.7 cm. The species does not appear to be of much economic importance.

Teredo samoaensis sp. nov.

Plate 10, figures 21–25

Shell similar to that of *Teredo furcillatus,* but generally more transparent, and with a smaller auricle. Interiorly the shell is characterized by a very broad, irregular apophysis, which is a useful, although not infallible guide in the identification of the shell.

Pallets with a stalk of medium length and a long tapering blade which is divided into two distinct portions. The basal portion, comprising about one-half the length of the blade, is broadly ovate and calcareous; the distal portion consists of a narrower, nearly straight-sided, more or less completely calcified semicylinder, flattened on the inner face, slightly cupped at the extremity. At the juncture of these two elements the pallet is encircled by a band of brown epidermis, which sometimes more or less completely envelops the distal portion.

The type specimen, from Tutuila, Samoa, is no. 1730, Museum of the California Academy of Sciences, San Francisco. Paratypes have been placed in the collections of the Department of Zoology, University of California, the Academy of Natural Sciences, Philadelphia, and the United States National Museum. The measurements of the type are: *shell,* height, 3.8 mm.; length, 3.8 mm.; *pallets,* length of blade, 2.9 mm.; width of blade, 1.3 mm.; length of stalk, 2.1 mm.

This species has been found only in Samoa, where it occurred commonly in the blocks from a board placed in November, 1923. This board was lost before adequate data were secured, and a second board placed the following June was not attacked at all by this species.

Teredo trulliformis sp. nov.

Plate 11, figures 31–34

Shell with a greatly reduced auricle, which is so fused with the posterior median portion that the boundary between the two can scarcely be detected on the exterior surface. Interiorly the auricle overlaps the posterior median portion a little distance, and its anterior edge can be distinguished, but does not form a shelf with a cavity behind it as it does in all of the foregoing species.

Pallets with a short, broad blade, and a stalk of medium length which, instead of tapering toward the end, becomes gradually expanded, like the handle of a trowel (Latin *trulla*). The distal portion of the blade is covered by a grayish or brownish epidermis, and the extremity is characterized by a shallow crescent-shaped excavation.

The posterior end of the tube is divided by a calcareous partition, forming two siphonal openings.

The measurements of the type are: *shell,* height, 3.4 mm.; length, 3.2 mm.; *pallets,* length of blade, 1.8 mm.; width of blade, 1.2 mm.; length of stalk, 1.3 mm. The type, from Honolulu Harbor, is no. 1731, Museum of the California Academy of Sciences, San Francisco. Paratypes are in the collection of the Department of Zoology, University of California, the Academy of Natural Sciences, Philadelphia, and the United States National Museum.

This species occurs commonly in the blocks from Honolulu Harbor, and somewhat rarely in the blocks from Pearl Harbor and Nawiliwili. The longest burrow recorded measured 9 cm.

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ERRATUM

Page 145, line 7 from bottom. *For* four *read* three.

EXPLANATION OF PLATES

PLATE 8

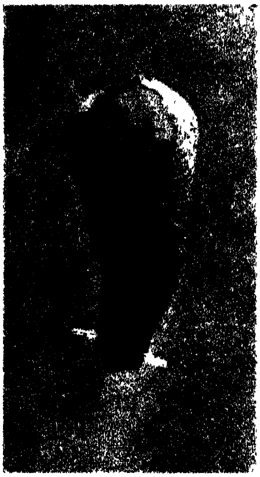
Fig. 1. *Martesia striata* from Pearl Harbor, dorsal view. × 2.

Fig. 2. Same, lateral view.

Fig. 3. Same, ventral view.

Fig. 4. Split section of a test block submerged five months at Cavite, showing *Martesia striata* in place in burrows. The burrows exhibiting a calcareous lining are those of *Teredo*. × 9/10.

Fig. 5. Portion of the surface of a block submerged nine months at Cavite, showing destruction by *Martesia striata*. × 9/10.



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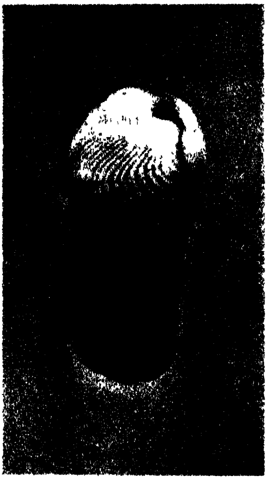

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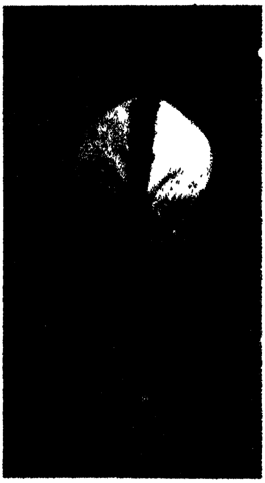

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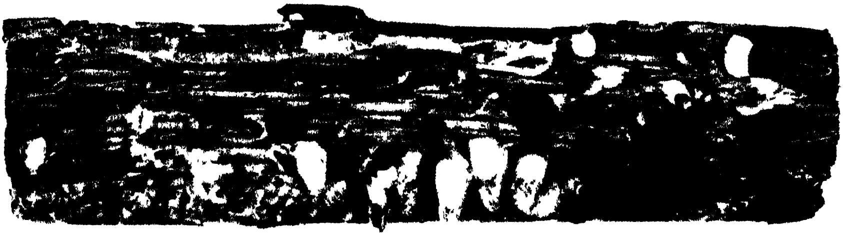

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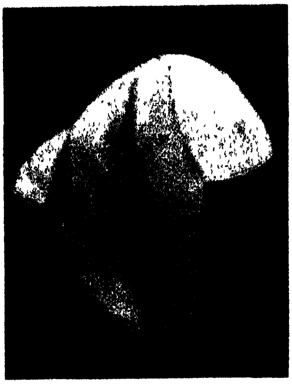
PLATE 9

Fig. 6. *Teredo parksi* from Pearl Harbor, exterior of right valve. × 7.
Fig. 7. Same, interior of right valve.
Fig. 8. Same, outer face of pallet.
Fig. 9. Same, inner face of pallet.
Fig. 10. Shells of *Teredo parksi* from Cavite. × 5.
Fig. 11. Pallets of *Teredo parksi* from Cavite. × 5.
Fig. 12. Shells of *Teredo parksi* from Honolulu Harbor. × 5.
Fig. 13. Pallets of *Teredo parksi* from Honolulu Harbor. × 5.
Fig. 14. Shells of *Teredo parksi* from Tutuila. × 5.
Fig. 15. Pallets of *Teredo parksi* from Tutuila. × 5.



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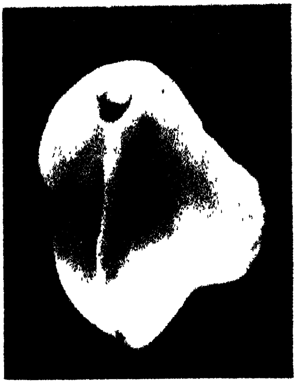

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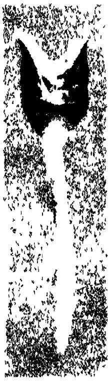

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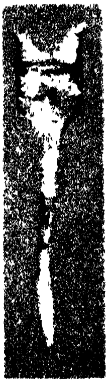

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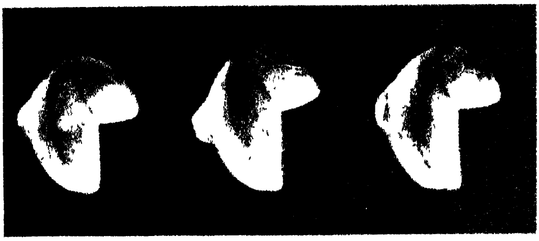

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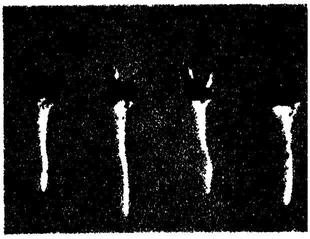

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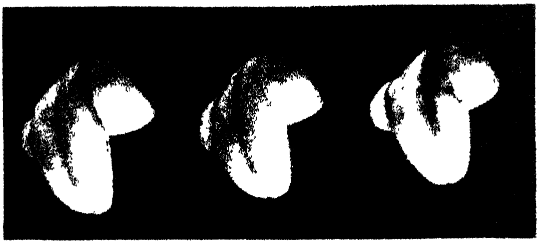

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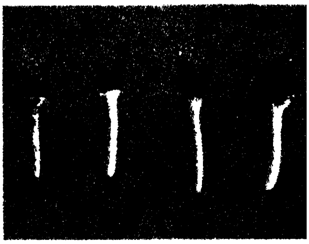

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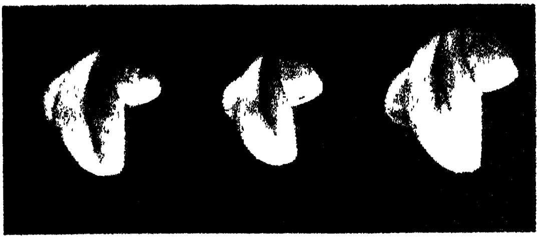

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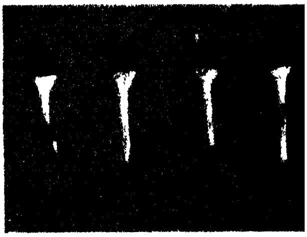

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PLATE 10

Fig. 16. *Teredo furcillatus* form Tutuila, exterior of right valve of the type. × 10.

Fig. 17. Same, interior of right valve.

Fig. 18. Same, outer face of pallet.

Fig. 19. Same, inner face of pallet.

Fig. 20. Series of pallets of *Teredo furcillatus* from Tutuila, selected to show variations. × 7.

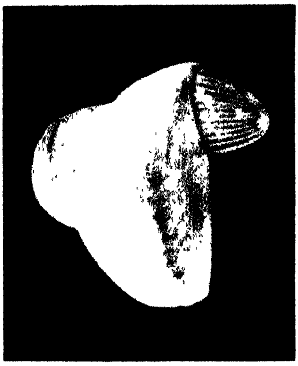
Fig. 21. *Teredo samoaensis* from Tutuila, exterior of right valve of the type. × 10.

Fig. 22. Same, interior of right valve.

Fig. 23. Same, outer face of pallet.

Fig. 24. Same, inner face of pallet.

Fig. 25. Series of pallets of *Teredo samoaensis* from Tutuila, selected to show variations. × 7.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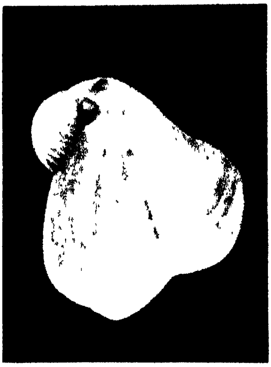
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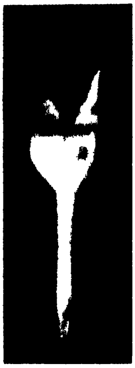
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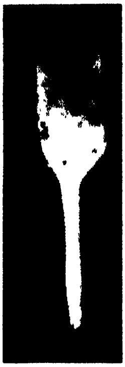
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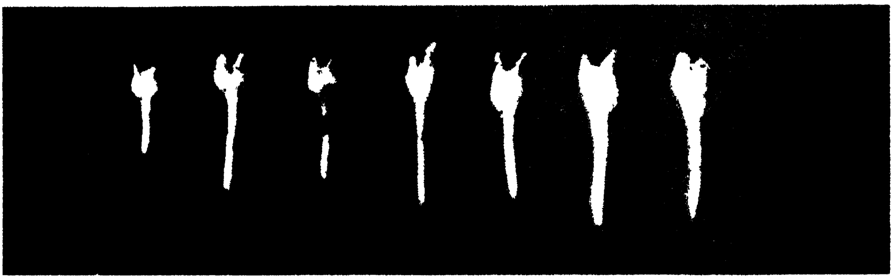
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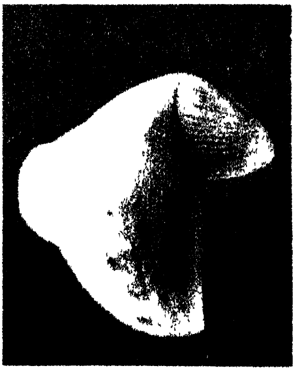
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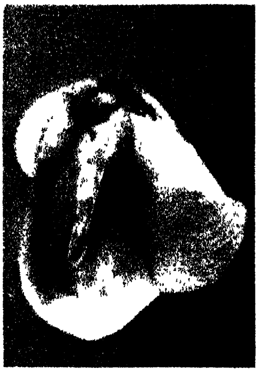
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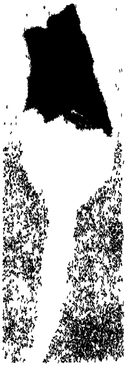
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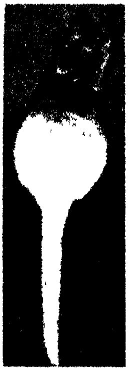
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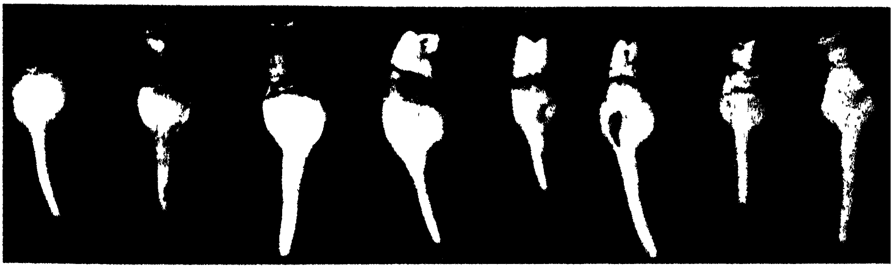
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PLATE 11

Fig. 26. *Teredo affinis* from Nawiliwili, exterior of right valve. × 10.

Fig. 27. Same, interior of right valve.

Fig. 28. Same, outer face of pallet.

Fig. 29. Same, inner face of pallet.

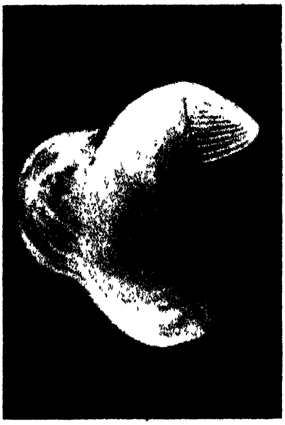
Fig. 30. Series of pallets of *Teredo affinis* from Nawiliwili, selected to show variations. × 10.

Fig. 31. *Teredo trulliformis* from Honolulu Harbor, exterior of right valve of type. × 10.

Fig. 32. Same, interior of right valve.

Fig. 33. Same, outer face of pallet.

Fig. 34. Same, inner face of pallet.



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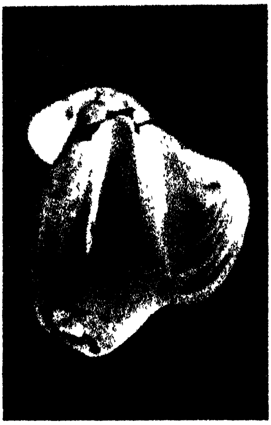

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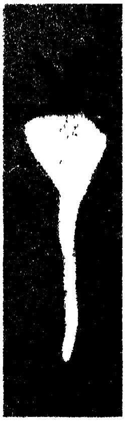

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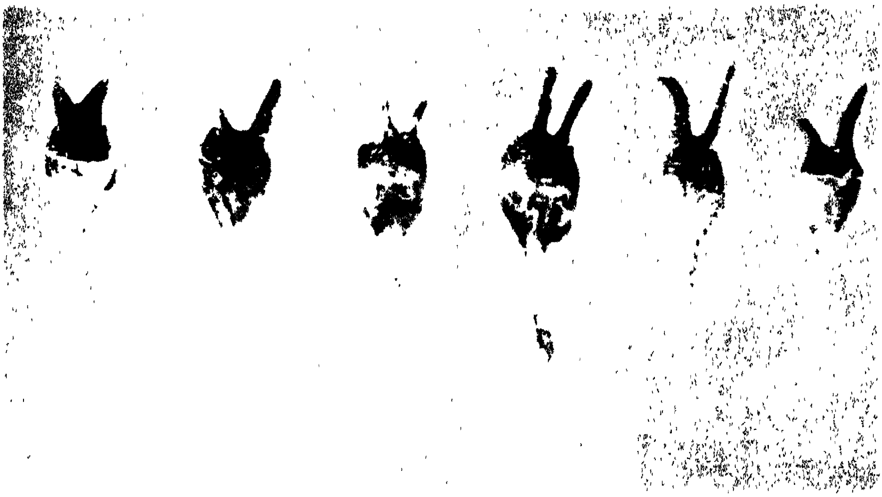

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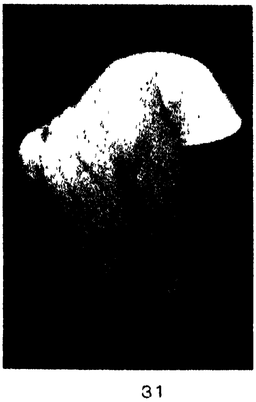

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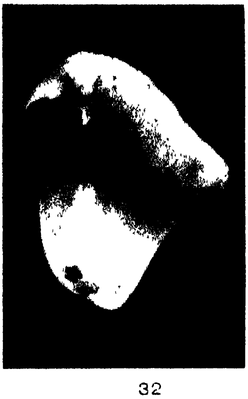

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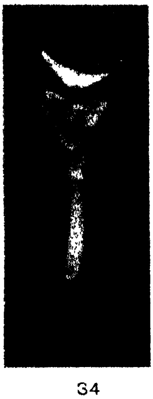

34

WOOD-BORING CRUSTACEA FROM HAWAII AND SAMOA

BY

ROBERT CUNNINGHAM MILLER

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY
Vol. 26, No. 8, pp. 159–164, plates 12–13
Issued May 29, 1924

WOOD-BORING CRUSTACEA FROM HAWAII AND SAMOA

BY

ROBERT CUNNINGHAM MILLER

The crustacean borers are represented in the test blocks from the islands by one species of the amphipod genus *Chelura*, and two species of the isopod genus *Limnoria*. Heavy attack by one or more of these species has occurred in the blocks from all stations mentioned in the preceding paper, with the exception of Nawiliwili, Territory of Hawaii, where the attack has been light, and Cavite, Philippine Islands, where no crustacean borers have been found.

Genus **Chelura**

Chelura insulae Calman

This species was described by Calman (1910) from Christmas Island in the Indian Ocean. It may readily be distinguished from the better known *Chelura terebrans* of Atlantic waters by the longer antennae and antennules, the enormously developed anterior gnathopods, and absence of the prominent spine on the third pleon segment which characterizes *C. terebrans*. In *C. insulae* the hind margin of the third pleon segment carries three short tubercles. The brush of hairs on the third uropods of the male is a further peculiarity of this species.

Chelura insulae occurs in great numbers in the blocks from Samoa, following up *Limnoria* and enlarging the burrows of the latter for its own occupancy. The organism apparently bores for food as well as shelter, as the passages it makes (pl. 13, fig. 8) are considerably larger than would be required for the latter purpose alone.

This species also occurs, though less numerously, in the blocks from Honolulu Harbor.

Genus **Limnoria**

Limnoria lignorum Rathke

This cosmopolitan pest occurs in considerable numbers in Pearl Harbor and, with *Limnoria andrewsi,* in Honolulu Harbor. A light and scattered attack by *L. lignorum* has occurred in the blocks from Nawiliwili.

The specimens from Hawaii are smaller than is usual for this species in our colder waters, averaging somewhat under 2.5 mm. in length. The length usually given for *L. lignorum* is 3 mm.; in San Francisco Bay the average length of adults of this species is 3.5 mm.

Limnoria andrewsi Calman

This species, like *Chelura insulae,* was described from Christmas Island by Calman (1910). It may be distinguished from *Limnoria lignorum,* as from the five other known species of *Limnoria,* by the relative shortness of the peduncle of the uropods; this in *L. andrewsi* is equal in length to or shorter than the outer ramus or exopod of the uropod, while in all of the other species the peduncle exceeds the exopod in length.

This species occurs in the blocks from Samoa in approximately the same numbers as *Chelura insulae.* The result of the combined action of the two is shown in plate 13, figure 6. *Limnoria andrewsi* also occurs with *L. lignorum* in Honolulu Harbor, where it greatly outnumbers the latter species, and at Nawiliwili, where both occur in limited numbers.

It is remarkable that, while *Limnoria andrewsi* is the dominant species in the test blocks from Honolulu Harbor, in the blocks from Pearl Harbor, only a few miles distant, *Limnoria lignorum* predominates to such an extent that if *L. andrewsi* occurs at all its presence has been overlooked.

The destruction of the test blocks by the crustacean borers has proceeded most rapidly in Honolulu Harbor (see pl. 13, fig. 7), where all three of the above-mentioned species occur together.

Zoological Laboratory,
University of California.

Transmitted March 20, 1924.

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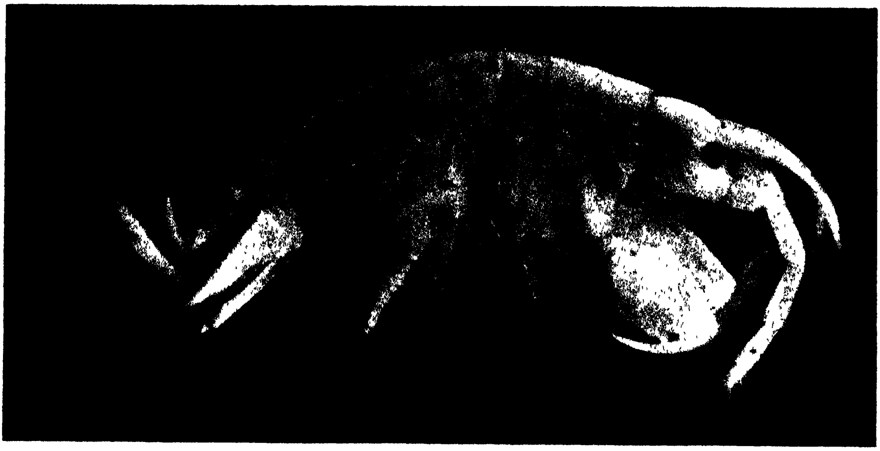
EXPLANATION OF PLATES

PLATE 12

Fig. 1. *Chelura insulae,* male, from Tutuila, Samoa. × 17.

Fig. 2. *Chelura insulae,* female, from Tutuila. × 17.

Figs. 3, 4, and 5. *Limnoria andrewsi* from Tutuila. Dorsal, lateral, and ventral views of female carrying advanced embryos in brood pouch. × 17.



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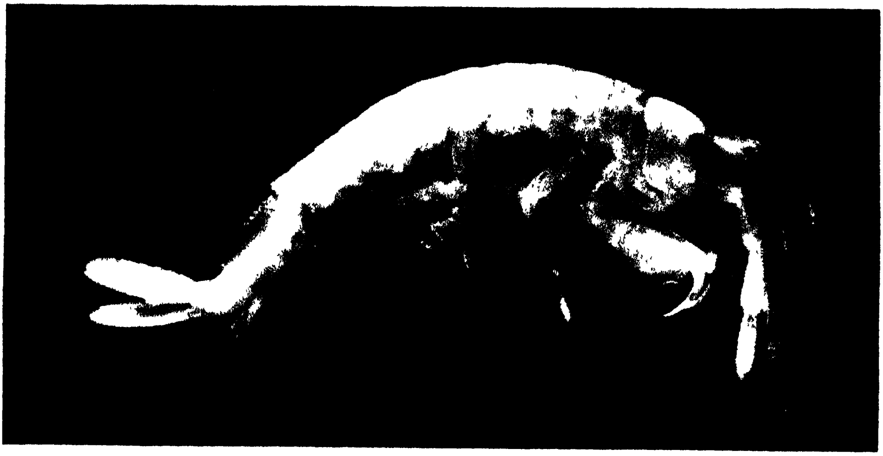

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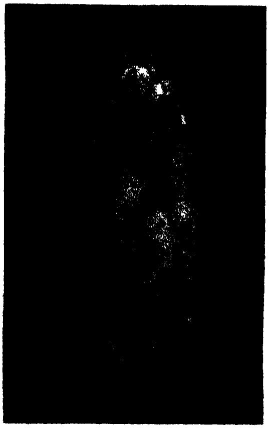

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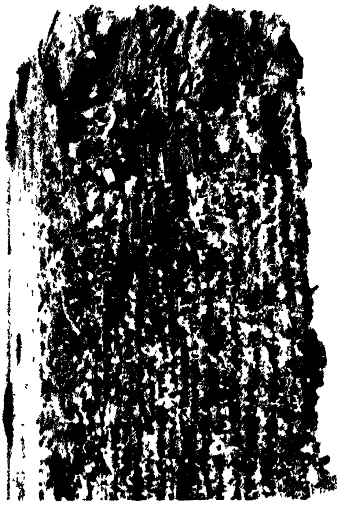
PLATE 13

Fig. 6. Portion of a 3½ in. by 3½ in. by 6 in. test block submerged eight months at Tutuila, showing damage by *Limnoria andrewsi* and *Chelura insulae*. The ends of a few *Teredo* burrows have been exposed. × ¾.

Fig. 7. All that remained of a 2 in. by 3½ in. by 5 in. test block submerged eight months in Honolulu Harbor. The major damage was occasioned by *Limnoria andrewsi; Limnoria lignorum* and *Chelura insulae* were also present in the block. All *Teredo* were dead as a result of exposure of their burrows by the crustacean borers. × ¾.

Fig. 8. Portion of block from Tutuila, showing *Chelura insulae* in place in burrows. One *Limnoria andrewsi* is seen in place near lower left corner. × 5.



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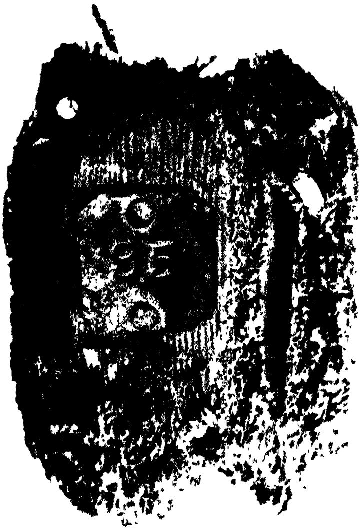

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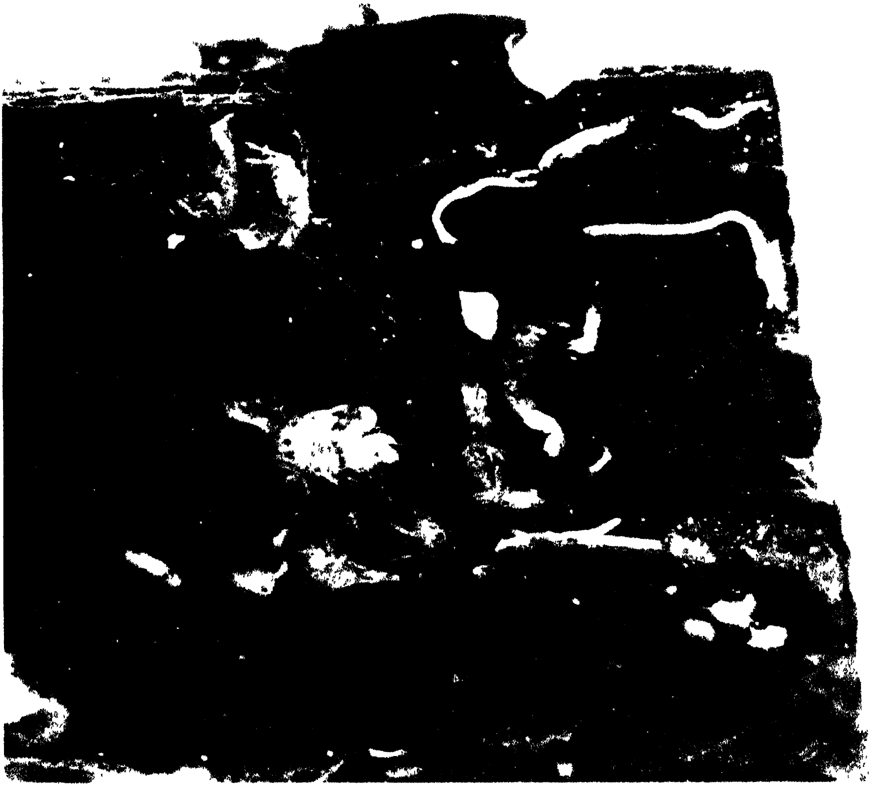

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THE CYTOLOGY OF ENDAMOEBA GINGIVALIS (GROS) BRUMPT COMPARED WITH THAT OF E. DYSENTERIAE WITH SPECIAL REFERENCE TO THE DETERMINATION OF THE AMOEBAS IN BONE MARROW IN ARTHRITIS DEFORMANS OF ELY'S SECOND TYPE

BY

CHARLES A. KOFOID AND OLIVE SWEZY

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Issued June 24, 1924

THE CYTOLOGY OF ENDAMOEBA GINGIVALIS (GROS) BRUMPT COMPARED WITH THAT OF E. DYSENTERIAE WITH SPECIAL REFERENCE TO THE DETERMINATION OF THE AMOEBAS IN BONE MARROW IN ARTHRITIS DEFORMANS OF ELY'S SECOND TYPE.

BY

CHARLES A. KOFOID AND OLIVE SWEZY

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INTRODUCTION

The tissue invasive potentialities of *Endamoeba dysenteriae* suggested by the occurrences of liver, lung, and brain abscesses of amoebic origin, have been further supported by the findings of Engman and Heithaus (1919) of amoebas in ulcers of the skin, since verified by us in their material and in one additional case, and by the discovery of amoebas in the testis by Warthin (1922) and in the semen by Hines (1923). Additional evidence indicative of the agency of the blood stream in the distribution of this species is offered in our findings (Kofoid and Swezy, 1922*a, b, c;* Kofoid, Swezy, and Boyers, 1922*a, b;* Kofoid, Boyers, and Swezy, 1922*a, b*; Kofoid, 1923) of *E. dysenteriae* in the bone marrow in cases of arthritis deformans of Ely's second type and in the inguinal gland in Hodgkin's disease. The relation of amoebiasis to iritis reported by Mills (1923) is of interest in this connection.

The apparent clinical relation between diseased teeth and arthritis and the occurrence of amoebas in the gums coupled with the conflicting opinions as to the occurrence of more than one species of amoeba in the mouth and tonsils, and the clinical reports of amoebas in malar abscesses, call for precision in the identification of the species of amoeba of the buccal and adjacent regions. This need is accentuated by the well-known resemblance of *Endamoeba gingivalis* of the mouth to *E. dysenteriae* of the colon in the matter of clear pseudopodia. In like manner the contested reports that *E. gingivalis,* as well as *E. dysenteriae,* ingests erythrocytes, and Dobell's (1919) atypical figures of nuclei of *E. gingivalis* strikingly resembling those of *E. dysenteriae,* add to the confusion, and to the desirability that investigations should establish more clearly the diagnostic differences between these two species and determine their local distribution in, and their respective relations to pathological conditions in the buccal, tonsilar, and malar regions of man. The present unsatisfactory state of our knowledge on these matters is reflected in the cautious statements of Fischer and Yü (1918) in their discussion of the amoeba of the oral region, and in the lack of evidence for Dobell's (1919) rather hesitant allocations of the amoebas included in his discussion of *E. gingivalis.*

The amoeba of the gums and tonsils of man acquired a temporary increase of interest about 1916 because of its supposed relation to pyorrhea alveolaris or Rigg's disease. This notoriety lapsed with the failure of treatment by emetin to relieve the symptoms permanently, or to destroy the organisms or prevent the recurrence of the amoebic infection about the teeth. A final decision as to the relation of this amoeba to pyorrhea may well be deferred until more extensive, critical experimental work has not only determined its relations, if any exist, to pathological conditions in the bucco-pharyngeal region, but has also discovered a successful treatment for the infection.

We have undertaken an investigation of *Endamoeba gingivalis* because of the possibility that it might enter the tissues or blood stream, localize in bone marrow, or otherwise become involved in the complex which results in some of the forms of arthritis. There is some morphological and some clinical evidence of a relationship of amoebas to Ely's (1920) second type of arthritis deformans; it is therefore a matter of clinical significance to determine whether or not *Endamoeba dysenteriae* is the only species concerned in this condition.

Since the species of amoebas are differentiated mainly and most decisively by cytological details, it is important that these features of the amoeba, or amoebas, of the bucco-pharyngeal region be clearly established. We accordingly present herewith the results of a renewed study of amoebas from about twenty different cases of oral infection, and portray the characteristic nuclear structures which enable one to distinguish this amoeba from *E. dysenteriae*.

ACKNOWLEDGMENTS

We are deeply indebted to Dr. L. W. Ely, of Stanford University Medical School, for his coöperation in supplying us with a portion of an excised head of a femur fixed on excision in formalin, and for a celloidin section prepared from the same specimen. This material came from case 187 of his second type of arthritis deformans (Ely, 1920). His courtesy has made possible a critical comparison of the amoebas of the bone marrow in his second type of arthritis with those of the buccal, or more specifically of the gingival region.

Acknowledgments for grants in support of this investigation are made to Mr. Franklin P. Nutting, Mrs. M. B. Fowler, the Carnegie Institution, the Board of Research of the University of California, the Carnegie Corporation, the California State Dental Association, and the Associated Radiograph Laboratories.

MORPHOLOGY

THE MOTILE PHASE

Endamoeba gingivalis has been found by us thus far only in the motile stages. The reports of Chiavaro (1914), Smith and Barrett (1915), Craig (1916), and Nowlin (1917*a*) of the finding of cysts appear to us to be erroneously based upon rounded-up individuals in which the ectoplasm is not demonstrable, or not readily seen, or in which it has been reduced to a film superficially resembling a cyst wall, but not forming one. We find no evidence for agreement with Goodrich and Moseley (1916) that Craig's cysts are those of a water amoeba.

The *motile phases* may be studied in activity on the warm stage for an hour or more in physiological salt solution, or better yet in saliva. Along the line of contact of the salt solution with iodine-eosin, the pseudopodia are fixed in normal contour with little or no contraction. Examinations of freshly made smears reveal many contracted individuals and others with numerous small pseudopodia. Emergence from these conditions into seemingly normal activity with single or few large pseudopodia can be followed on the controlled warm stage. There is abundant evidence of a great variety of appearances of the pseudopodia in response to conditions of shock, temperature, and possibly of changes in pH. Smears fixed under different conditions reveal differences in number, shape, and extent of pseudopodia, and in the sharpness of delimitation of ectoplasm and endoplasm. Smears from the same region of the gums may exhibit, in different slides, marked differences in the relative extent of the ectoplasm due to some undetermined factor. These differences may have been the occasion for the concept of more than one species of amoeba in the mouth, resulting in the description of *E. pyogenes* Verdun and Bruyant (1907*a, b*), *E. confusa* Craig (1916), and *E. macrohyalina* Tibaldi (1920). The contractile vacuole reported by Tibaldi may have been only a discharging food vacuole.

ECTOPLASM

When in locomotion *Endamoeba gingivalis* normally exhibits both in life and in stained preparations a division of its substance into a clear, peripheral ectoplasmic zone and a granular, more deeply staining endoplasm containing food vacuoles and nucleus. In life the ectoplasm is crystal clear and hyaline. In material stained in Giemsa the ectoplasm retains a bluish tinge and rarely shows differentiation. In iron-haematoxylin it generally shows a clear margin and a very faintly granular and alveolar central area (pl. 16, figs. 17–19). This area is sharply differentiated from that of the granular endoplasm, which is more deeply stained, with darker and coarser granules. The line of demarcation between ectoplasm and endoplasm is, as a rule, both in life and after staining, sharply delimited, but there are amoebas in which the transition is somewhat graduated (pl. 16, fig. 17). Others, in the non-motile phase, may exhibit no ectoplasm at all (pl. 15, figs. 8–11). The sharpness of the delimitation between ectoplasm and endoplasm can be seen only in transverse optical section. Focus at tangential levels obscures it.

Moribund amoebas are evidently not uncommon as is shown by absence of food vacuoles, abnormal vacuolation, and loss of stainability of the nucleus (pl. 15, fig. 14). There are, however, seemingly normal amoebas exhibiting none of these conditions in which no peripheral ectoplasm can be differentiated. The normally constituted endoplasm in such amoebas extends to the pellicle. It seems that under certain conditions the endoplasm may normally invade and intermingle with apparently the entire ectoplasmic region except the pellicle. Craig (1916, pl. 6, figs. 9–12) has described some of these individuals when freed from vacuoles and rounded up as precystic and cystic forms. The evidence for his conclusion is unconvincing, beyond the analogy that *E. dysenteriae* in precystic and cystic stages frees itself from vacuoles and no longer shows an ectoplasmic region. We find no evidence that these stages form cyst walls, glycogen vacuoles, and chromatoidal bodies, and then pass through mitoses as in the formation of cysts of *E. dysenteriae.* Feeding and metabolism are evidently slowed down, possibly as in depression periods in *Paramecium.* If so, death, or recovery of normal activities, are the alternatives.

The relative amount of the ectoplasmic territory in active amoebas may be large. In a slide full of amoebas in pseudopodial activity in a rounded-up individual free from food vacuoles with a clear ectoplasmic zone 2.7 microns thick and a total diameter of 18 microns, the ectoplasm formed 55 per cent of the volume. In most cases the relative volume of the ectoplasm is much smaller.

A striking characteristic which seems hitherto to have been wholly overlooked is the *ectoplasmic pellicle* (fig. A, *pell.*) which may be found in practically all individuals when properly stained in iron-haematoxylin and erythrosin. It is a thin, homogeneous, more deeply stained surface film, uniform in thickness in the iron-haematoxylin alone, while in the counterstain it stains more deeply pinkish than the underlying ectoplasm. It covers the entire surface uniformly, including the pseudopodia, and appears on individuals showing no other ectoplasm. It is evident only in optical transection and disappears in tangential focus. We can find no such structure in *E. dysenteriae* in the motile phase.

PSEUDOPODIA AND LOCOMOTION

The *pseudopodia* of *E. gingivalis* vary in size, number, and shape. They often have only a single large pseudopod (fig. A; pl. 14, figs. 2, 3, 5) whose base is 0.5 to 0.9 the diameter of the body, and whose length only occasionally exceeds the width of the base. They are usually symmetrically rounded in outline but when fully formed may become inflated distally (fig. A; pl. 14, figs. 1, 3) or slightly asymmetrical. During pseudopodial activity the pseudopodia may be formed in such rapid succession that two to five are in process of formation or retraction at one time.

Active locomotion in one direction is accomplished by the repeated formation of pseudopodia in one region in rapid succession. One amoeba, 20 microns in length, was observed to traverse a microscopic field, and turn and retraverse it, a total distance of 386 microns in eight and one-half minutes. This amoeba had been freshly mounted in saliva but had not been placed on a warm stage. The formation of pseudopodia may continue in rapid succession with no locomotion at all. One amoeba while almost stationary thrust out twenty-four successive pseudopodia in two minutes. No conclusive evidence of a permanent polarization has been found by us. Prolonged locomotion in a single direction can often be seen, and repeated successive formation of pseudopodia at or near one pole, with an approximation of equality in the number of pseudopodia formed at the right and left which might suggest polarity and a spiral organization and behavior; but this is frequently interrupted, perhaps by a motor reaction. These interruptions taken in connection with the not infrequent total reversal of the direction of locomotion make it difficult to posit either permanent polarity or a spiral organization in this amoeba, such as Schaefer (1923) has detected in free-living amoebas. The occasional adhesiveness of the posterior end suggests an attachment cone (*att. c.*, fig. A; pl. 15, fig. 6).

Amoebas freshly mounted in physiological salt solution may show very many small pseudopodia, sometimes clustered (pl. 14, fig. 1) possibly in the period of recovery from shock. The single pseudopodium—or at the most two or three—of the broad, symmetrically rounded type, rarely very much longer than the width of the base, seems to be the normal form both in locomotion and in the stationary phase. It is very often found in smears in saliva that have been allowed to stand for a short time before fixation.

The pseudopodium is formed by localized solation followed by sudden spread of the area and abrupt expulsion of the liquefied ectoplasm, which gels suddenly when the pseudopodium has been completed. There is no corresponding abruptness in the movement of the endoplasm. We can find no evidence of any constancy in the position of the nucleus with relation to the area of origin of the pseudopodia or to the direction of locomotion. It is detected and followed with difficulty in life and is found in the widest possible range of location in fixed amoebas, though, in the majority of instances, it is subcentral.

ENDOPLASM

The *endoplasm* is rather coarsely alveolar and more often than not in normal individuals is crowded with food vacuoles (pl. 16, fig. 15). In life it is sharply delimited from the very hyaline ectoplasm by its granular composition and food vacuoles, both of which are even more clearly demonstrated by the staining process. The living endoplasm is very mobile, as is indicated by the shifting of food vacuoles and of the nucleus as well as by its adjustments to pseudopodial formation and to locomotion. It may fill the entire body out to the ectoplasmic pellicle, seeming to obliterate the ectoplasm, especially in rounded-up and possibly also in moribund individuals. In other amoebas with normal food vacuoles and nuclei, but more or less rounded-up, there may be no demarcation, and the endoplasm may fill the body to the pellicle. The occurrence of amoebas, some with, and some without ectoplasm, in adjacent areas in the same preparation suggests the action of some internal factor of metabolism or possibly of senescence or depression in certain amoebas. Individuals without differentiated ectoplasm occur also in *E. dysenteriae*.

The endoplasm of *E. gingivalis*, even when free from food contents, is very noticeably vacuolated, as compared with the endoplasm of the motile *E. dysenteriae* from the colon of man and of the cat. This is not a mere protoplasmic alveolation but the formation of spherical, fluid-filled vacuoles which crowd the endoplasm often to its margin. They appear to grade into the food vacuoles both in size and in contents and may thus represent the last stages of the intracytoplasmic digestion of the contents of the food vacuoles. Inspection shows that the solid contents, both bacterial and non-bacterial, of the smaller vacuoles are less dense and less deeply stained than those of the larger ones, and leads to the inference that the fluid-filled vacuoles which grade down in size represent the later phases of the incorporation of ingested food.

In stained preparations instances are frequently seen of amoebas from which the liquefied chromatin of an ingested leucocyte is ejected in a viscous thread through a circular pore-like area in the cytoplasm, thus emptying the vacuole of these food remnants (pl. 17, figs. 44, 45, 49, 50). The normality of this phenomenon is still to be determined.

ORGANELLES OF THE ENDOPLASM

The *food vacuoles* are the most evident and striking feature of *E. gingivalis*, mainly because of the size of the vacuoles, their siderophile contents, and their number. They thus constitute a considerable part of the volume of the body, computed, in one case as 35 per cent. These vacuoles are spherical, or spheroidal, sometimes slightly deformed (pl. 14, fig. 4) by mutual pressure or contact with the endoplasmic periphery or by contact with the nucleus, although they appear more often to compress it. They are filled by a clear non-staining fluid in which the food body floats in a central position, or near the center of the mass in the deformed vacuoles. The volume of the fluid is very much greater than that of the enclosed food body, computed as from two to ten times as great in the vacuoles containing leucocytes, and as much as twenty-five times greater in some vacuoles containing bacteria.

The *food bodies* are characteristically of two kinds, leucocytes and bacteria. We have not seen epithelial cells nor erythrocytes in the vacuoles. It seems neither usual or customary for amoebas to ingest the erythrocytes. We infer that Goodrich and Moseley's (1916) statement of their presence in *E. gingivalis* may be based on the occurrence of rounded-up nuclei of leucocytes devoid of cytoplasm in the food vacuoles, whose size and appearance resemble that of plasmolyzed and digesting erythrocytes. The findings of Bass and Johns (1915) tend to support the occurrence of the erythrocytes in so far as they find certain vacuoles with a food body staining pink in carbol-fuchsin and methylene blue while the larger and less regular bodies stain blue. This difference is in our judgment due to a change in chemical state resulting from the progress of digestion rather than to an initial difference in the food body. The grounds on which our conclusion rests are: (1) the entire absence of structurally recognizable red cells in freshly examined living amoebas and in stained preparations; (2) the pink-staining bodies are in the smaller and older vacuoles; (3) the larger blue-staining bodies when not fully liquefied show traces of nuclear organization and the notch or cleft with the extranuclear centrosome (pl. 18, fig. 49); and (4) the other and in some instances progressively smaller vacuoles are transition stages from normal leucocyte nuclei to homogeneous spherules of deeply staining fluid of nuclear origin.

However, Smith, Middleton, and Barrett (1914) state that they had noted the rapid hemolysis of a freshly englobed red blood cell which disappeared by solution without fragmentation in five minutes, and add that Smith and Barrett had seen the same phenomenon in endamoebas from pyorrheal pockets. Should this be normal and usual the disagreements as to the ingestion of erythrocytes would be more probably of observation rather than of fact. The extent to which *E. gingivalis* utilizes erythrocytes as food and the conditions under which it does so await further evidence. Such utilization appears to be slight or accidental rather than customary.

The nature and condition as well as the types of the leucocytes ingested by *E. gingivalis* are all obscured by the fact that the food bodies are obtained in an area of exudation where bacteria and cellular detritus abound. This problem can be attacked by an analysis of the amoebas *in situ* in the gums, but our available material at present is limited to smears only from the gums. In these the relationship of the amoebas to the tissues, and the distribution and types of leucocytes in the vacuoles and in the gingival area are disturbed by removal and smearing.

The preparations show as a rule only nuclei or their degeneration products in the food vacuoles, not normal leucocytes with cytoplasm. Only occasionally does one find evidence of a protoplasmic film around the nucleus. This may be because of the prompt digestion of the cytoplasm or of the ingestion of naked nuclei from the cellular detritus which abounds in the smears. The condition of the nuclei in the vacuoles and also in the detritus is such that identification of the type of leucocytes to which they belong is more or less conjectural. Many nuclei in the food vacuoles still show the bay or cleft which may even contain a granule in the cytoplasm which appears to be the centriole (pl. 18, fig. 49). Such nuclei appear to belong to the transitional, mononuclear type. Some nuclei resemble those of the lymphocytes, and once in a while one sees deeply lobed nuclei of the polymorphonuclear type (pl. 18, fig. 44). Still less frequent are vague networks suggesting the nuclei of mast cells. The larger nuclei of epithelial and other human cells have not been found in the food vacuoles.

The food vacuoles enclosing bacteria usually contain but a single organism. Clustered groups occur, especially of the smaller bacteria, but they are rare. The vacuole conforms in contour to the shape of the ingested organism. *Micrococcus* lies in spherical vacuoles, *Bacillus* and *Bacterium* in elongated ones, and *Streptococcus* in ellipsoidal ones. Although elongated bacteria such as *Leptothrix* and *Spirochaeta* are abundant in our preparations they have only rarely been detected in the food vacuoles of the amoebas associated therewith (pl. 18, fig. 51). The fine, deeply staining threads sometimes found coiled in the cytoplasm of the amoebas are in some instances demonstrably the filamentous remnants of the homogeneous, viscous substance of food bodies of leucocytic origin retained in the cytoplasm after ejection of the main mass. We infer that others of similar appearance, but not with an enveloping vacuole and having no present connection with any vacuole, are intracytoplasmic remnants of the viscous thread formed at ejection and entangled in the cytoplasm (pl. 18, fig. 46).

There are amoebas feeding solely on leucocytes (pl. 16, fig. 18) and others with bacteria only (pl. 18, fig. 51) in their vacuoles, while a few contain both. The large numbers of bacteria figured by Mendel (1916) appear to be over- or underlying rather than ingested organisms. Preparations made by the wet-smear method do not confirm the presence, normally, of such large numbers of bacteria. A survey of amoebas from three different cases repeatedly examined, together with numerous isolated preparations, is convincing that *Endamoeba gingivalis* is primarily a feeder upon nuclei and cells of its substrate and only secondarily upon the bacterial milieu with which it is associated. Although bacteria in considerable variety and very great abundance are continually present around them, the amoebas have in their food vacuoles nuclei rather than bacteria. When bacteria are present their number is generally so insignificant as to give the impression of contamination rather than selection.

The relative volumes of food of nuclear and of bacterial origin stand in still sharper contrast, the nuclear material appearing to be many thousand-fold greater than the bacterial material. The duration of the intravacuolar status of the two kinds of food may favor a larger estimate of the total bacterial contribution; but the trend of the evidence is to the conclusion that the amoebas feed selectively on the leucocytes and the nuclei of leucocytes to the exclusion of those of other cells, and that bacteria are not the main food and may be largely contaminative rather than selected.

The extrusion of food bodies of nuclear origin is seen not infrequently in smears. This appears to occur only in nuclei which have been liquefied by the digestive process and reduced to a homogeneous, viscous, deeply staining, siderophile fluid. Comparison of a number of such food bodies indicates a progressive disappearance of the chromatin network and the dissolution of the chromatin and nuclear sap into a substance of more or less homogeneous appearance. This sometimes shows a concentric stratification with a narrow outer, more deeply staining shell, a clear narrow zone within this, and a homogeneous central mass, less deeply stained (pl. 18, fig. 49). These are so frequently observed in heavily destained smears that they are to be interpreted as results of metabolism and not artifacts of the staining process.

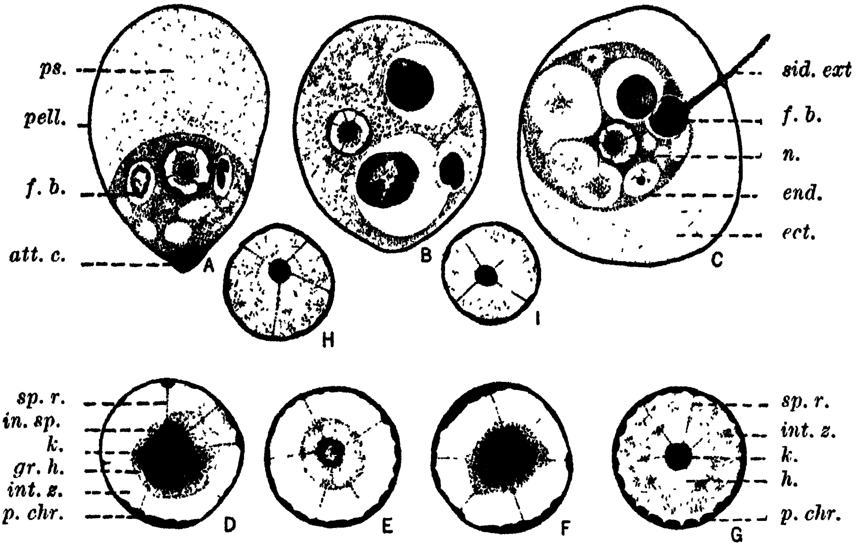
This homogeneous substance is ejected from certain vacuoles of some amoebas in our smears. It occurs from a *single vacuole only*, as a rule, and the material ejected comes mainly from the food body rather than from surrounding fluid. It is ejected along a canal through the endoplasm and ectoplasm, if present, and through the pellicle. At the point where it traverses this layer a deeply staining ring is formed surrounding the opening (pl. 18, figs. 44, 45). There is no evidence that this canal and the ring are definite or permanent organs. They appear to be temporary, and in the case of the ring to be the result of the local condensation of the pellicle. The substance is shot out or dragged out in long, tenuous strings, which may be several diameters of the amoeba in length. They resemble in stainability and physical appearance the strung-out degenerating nuclei of the gingival exudate often seen in smears. The substance is evidently remarkably viscous, and resistant to dissolution. Strands from adjacent amoebas coalesce in some instances (pl. 18, figs. 44, 45). In one instance (pl. 18, fig. 46) the thread seems to have ricocheted through the cytoplasm during the process. The same amoeba exhibits an *endoplasmic* protrusion through the ring in the pellicle, which persists after the disappearance of the food body.

It is probable that this phenomenon is a consequence of the smearing process. It affects, however, but a single food body, as a rule, and occurs also in amoebas that are imbedded in bacterial masses. These masses show no immediate effects of disturbance, and the amoebas with the extrusion show no more distortion than others without it. It is possible that the phenomenon results from contractions of the amoeba rather than from external compression.

NUCLEUS

The *nucleus* of *E. gingivalis* is its most distinguishing characteristic and conclusively demonstrates its specific distinctness from *E. dysenteriae* and all other amoebas of man. The nucleus of *E. gingivalis*, in common with those of other species, is spherical in form but is subject in this species to a greater proportion of minor irregularities in contour than is usual in other species. In some instances these are clearly the result of pressure of adjacent food vacuoles. In other instances the existing contacts do not suggest such pressure. There is thus either a greater crowding of food vacuoles in *E. gingivalis*, or a greater pliability of the nucleus in this species than in others in which its sphericity is more uniformly maintained.

The *diameter of the nucleus* varies from 1.8 to 5.6 microns in 75 amoebas from various preparations from three different persons. There is a little evidence of a difference in size in the three cases. In a case in which there is no history of pyorrhea, no devitalized teeth, and only a few fillings, the diameters of 25 nuclei range from 1.8 to 4.4 and average 2.93 microns in 25 amoebas whose mean diameters range from 9.1 to 17.7 and average 12.7 microns. The amoebas in this case are few in number and of small size with larger ones on some days.



Figs. A–F. *Endamoeba gingivalis* (Gros, 1849) Brumpt, 1913, from smears of gingival exudate fixed in Schaudinn's fluid and stained in iron haematoxylin. Camera lucida drawings, figures A–C × 1750; figures D–F × 3500. Abbreviations: *att. c.*, attachment cone; *ect.*, ectoplasm; *end.*, endoplasm; *f. b.*, food body; *gr. h.*, granular halo; *h.*, halo; *in. sp.*, inner sphere; *int. z.*, intermediate zone; *k.*, karyosome; *n.*, nucleus; *p. chr.*, peripheral chromatin; *pell.*, pellicle; *ps.*, pseudopodium; *sid. ext.*, siderophile extrusion; *sp. r.*, spoke radii.

Fig. A. Motile amoeba with single pseudopodium and posterior attachment cone. Fig. B. Rounded-up amoeba with no ectoplasm, three food vacuoles, one with a lymphocyte and one with a polymorphonuclear leucocyte. Fig. C. Rounding-up individual with large amount of ectoplasm, food vacuoles with leucocytes or remnants of them. The siderophile contents are being extruded as a viscous strand through the ectoplasm and a stained ring in the pellicle. The outer end is disintegrating.

Fig. D. Nucleus with clustered karyosomal granules, in a darker central region, distinct granular halo, and irregular peripheral chromatin. Fig. E. Heavily destained nucleus with more regular peripheral chromatin, granular peripheral ring about the halo, two small karyosomal granules in a darker central region. Fig. F. Not heavily destained, irregular peripheral chromatin, three karyosomal granules in darker central area.

Figs. G–1. *Endamoeba dysenteriae* (Councilman and Lafleur) × 3500. Fig. G. Nucleus from motile amoeba in human stool. Note clear halo and granular intermediate zone. The halo in figure G is exceptionally large and its chromatin less regular than usual. Figs. H and I. From motile amoebas in human bone marrow in the excised head of the femur fixed in formalin, but later refixed in Schaudinn's fluid, decalcified, and stained in iron-haematoxylin, from Ely's case 187 of arthritis deformans. The halo is difficult to demonstrate in these amoebae and is generally small, or absent as in figure I.

In a second case, also with no history of pyorrhea, no devitalized but a number of filled teeth, the diameters of the nuclei range from 2.4 to 4.0 and average 3.34 microns in 25 amoebas whose mean diameters range from 12 to 20.9 and average 16.2 microns. Preparations made 10 years prior to those from which these measurements were taken show a range in the diameters of the nuclei of 3.2 to 4.8 with an average of 3.7 microns in 25 amoebas with mean diameters of 10.8 to 20.0 and an average of 15.3 microns.

In a third case in which there is a history of pyorrhea for thirty years with recurrent formation of pyorrheal pockets, six devitalized teeth remaining and four extracted, and a number of fillings, the diameters in 25 nuclei range from 3.0 to 5.6 and average 4.01 microns in 25 amoebas whose mean diameters range from 11.3 to 21.6 and average 16.5 microns. The amoebas are abundant in this case.

There is thus an increase in size of about 30 per cent in the diameters both of the nucleus and of the whole amoeba in this case of long standing infection as compared with that of a light infection with more normal gums in the first case.

The nucleus (fig. D) consists of a membrane (*n. m.*), peripheral chromatin (*p. chr.*), intermediate zone (*int. z.*), granular halo (*gr. h.*), a central darker area or inner granular sphere (*in. sp.*), karyosome (*k.*), spoke radii (*sp. rad.*), and nuclear fluid.

The *nuclear membrane* is a thin homogeneous sheet of uniform thickness, visible in some preparations (pl. 15, fig. 13; pl. 17, fig. 29) in optical section as separate from the peripheral chromatin applied to it, by its lighter stain. The chromatin of the nucleus of *E. gingivalis,* as in other amoebas of man, is gathered in two regions of the nucleus, namely, in the peripheral zone on the inner face of the nuclear membrane, and in the karyosome.

The *peripheral chromatin* (*p. chr.*) is best seen in optical section at the equator of the nucleus. In *E. gingivalis* it is generally found in a beaded state, or in larger aggregates, with the scattered masses of chromatin flattened against the membrane and *convex* inwardly. Only rarely (pl. 17, fig. 23) does it approach the condition of a continuous, uniformly thick layer spread over the membrane throughout. Even in such cases sharp definition will sometimes reveal slight traces of clumping. The peripheral chromatin is markedly uneven and irregular in distribution. The clumps are, as a rule, localized in one or two regions in the section, and dispersed and irregularly scattered in the other arcs as originally described by

Chiavaro (1914). They also vary greatly in diameter, thickness, and volume. As a result of these irregularities, the nuclei often have an appearance, under low magnification, of incompleteness. In comparison with those of *E. dysenteriae* they are thus much less regular in structure and appear to be sparsely supplied with peripheral chromatin (pl. 17).

A comparison of a large number of nuclei in a number of different preparations gives the impression that in many instances the chromatin tends to mass on the areas least in juxtaposition to the food vacuoles, or that the areas nearest the vacuoles have least peripheral chromatin. This relation is not universal but predominates generally when tested, suggesting chromatin reduction in the region of contact with metabolic activity.

The *intermediate zone* (*int. z.*) is a wide clear region lying between the peripheral chromatin and the karyosome about 0.25 nuclear diameter in width and containing little or no substance stainable in either iron-haematoxylin or Giemsa. This area of unstained nuclear sap is traversed by a varying number of delicate filar or granular *spoke radii* (*sp. rad.*) which connect peripheral clumps of chromatin with the granular halo about the karyosome, especially with its projecting angles, and pass through this to the karyosomal granule or granules. Their position is normally radial but in some instances they are deflected from this course.

The central, more deeply staining mass in *E. gingivalis* is divisible into an outer granular halo (*gr. h.*), a karyosome, and an inner granular sphere (*in. sp.*) around the karyosome or cluster of karyosomal granules. This mass is central or subcentral in location. It is even more uniformly central than is the karyosome of *E. dysenteriae in the motile state* but the karyosome within it is much more variable in position than that of *E. dysenteriae* with respect to its halo. In the more stabilized conditions of the cyst after mitoses are completed the karyosome of *E. dysenteriae* is normally central in nucleus and halo. In the motile phase it is much more variable in position and frequently off center in all preparations accessible to us (pl. 17, figs. 32–43).

The structure of the *granular halo* in *E. gingivalis* (pl. 17, figs. 20–31) is variable, in part as the result of decolorization, and in part probably in consequence of metabolic conditions. It is a very large body whose diameter is approximately half that of the nucleus. Its contour is irregularly polygonal with projecting angles passing into spoke radii, or subspherical, or ellipsoidal. By destaining it is resolvable into an irregular granular structure in which there are traces of a mesh or alveolar sponge work. This peripheral mass is fairly uniform in consistency within itself except for its margin, which may be more deeply stainable (pl. 17, fig. 28), and except for the inner sphere around the karyosome, which is more or less indistinctly differentiated but varied in appearance according to the amount of destaining. If destaining is prolonged the inner sphere fades into a vague cloud with a few darker peripheral points. The intermediate zone surrounding it is completely destained, except for the spoke radii, when the stain in the granular halo is sufficiently reduced to reveal the enclosed karyosome. If the halo is but slightly destained the karyosome is detected within it with difficulty. The meshwork is fairly uniform throughout. In no case do we find it in well stained preparations extending to the periphery as figured by Leyden and Löwenthal (1905) and reproduced by Doflein (1916, fig. 682C). In heavily destained nuclei we find in optical section a circular peripheral boundary as in Doflein's figure B. These figures and those of Hartmann (1910, fig. 5, reproduced in many German texts) fail to make the distinction between the outer clear zone on the one hand and the granular or alveolar zone of the halo on the other. Chiavaro's (1914) figures faintly portray the halo and one to three granules. For a clear understanding of the differences between *E. gingivalis* and *E. dysenteriae* it is essential to determine sharply these outer limits of the granular halo and its relations to the karyosome. These differences can be clearly brought out by controlled decolorization.

The *karyosome* is a deeply staining homogeneous granule, or a group of two or three loosely agglomerated bodies of spherical or irregular shape. Their location is central, subcentral, or in some instances excentric (pl. 17, figs. 20–31). When a single body is present it is often spherical; but ellipsoidal, irregular, and asymmetrical forms also occur, especially in the clustered granules. In stainability these bodies resemble the karyosome of *E. dysenteriae.*

An inspection of published figures of the nuclei of *E. gingivalis* (Prowazek, 1904; Leyden and Löwenthal, 1905; Bass and Johns, 1915; Chiavaro, 1914; Hartmann, 1910; Brumpt, 1913; Smith and Barrett, 1915; Craig, 1916; Mendel, 1916; Goodrich and Mosely, 1916; Goodey and Wellings, 1916; Nowlin, 1917*a;* Dobell, 1919; Hartmann and Bělař, 1921, and Nöller, 1922) convinces us that these figures are inadequate. A differential diagnosis from *E. dysenteriae* on nuclear structure is difficult if not impossible from these figures. All figures prior to those of Dobell give so little detail and are on so small a scale that the finer structure is not critically revealed, while the two figures of Dobell (1919, pl. 5, figs. 93 and 94) are not typical of the material at our disposal and rather closely resemble those of his and of our figures of *E. dysenteriae.* He figures (his fig. 94) a thick, heavy, homogeneous, uniform layer of peripheral chromatin, or one only very slightly indented (fig. 93). The karyosome is figured by him as solid and spherical (fig. 94) or ellipsoidal (fig. 93); but such irregularities as appear in these outlines seem to be caused by the coarse mesh of the half-tone. He states that the karyosome is spherical, and characteristically expresses his belief that Prowazek's (1904) finding of several separate granules is incorrect, citing the fact that he, himself, had not so found conditions in normal individuals. In our material which has been promptly fixed and carefully decolorized, with cytoplasm, food vacuoles, and nuclear structure of the general and supposedly normal type, the somewhat irregular or subdivided karyosome is of very general occurrence. Dobell admits (p. 95) that he had studied only a limited amount of material.

His figures differ also in another feature from our findings (pl. 17, figs. 20–31), namely, the presence of a very narrow, *clear* halo around the karyosome, with a faintly stained vaguely structureless intermediate zone. We find, as a rule, a granular, stainable halo with rather more definite peripheral margins. It is surrounded by a colorless or less granular or less stainable intermediate zone, and is, relatively, decidedly wider than the halo Dobell found. On the whole, his figures are rather strikingly similar to those of the nuclei of motile *E. dysenteriae* on his plate 5. This similarity has not been found by us to be usual or prevalent in any cases of infection or preparations of *E. gingivalis*, nor does it appear in the previously published figures of others. The nearest approach to his type is seen in our plate 17, figure 31, the nucleus from a small amoeba.

Dobell's figures also lack spoke radii which a skilfully used binocular brings to light in this species.

In this comparison we infer not that Dobell has figured *E. dysenteriae,* but rather that the nucleus as drawn by him is not, in a single structural feature, typical of the nucleus of *E. gingivalis* when critically resolved with the binocular in preparations made immediately by the wet-smear, Schaudinn, iron-haematoxylin method.

When viewed with low oculars, well-decolorized nuclei give a vague picture which resembles the vacuolated zone about the karyosome in the widely copied figures of Leyden and Löwenthal (1905). This appears to be based on the spoke radii and the denser sphere of granules on the periphery of the granular halo. Resolution under optimum optical conditions reveals the structures figured by us.

The *homologies* of the nuclear zone in *E. gingivalis* with those of *E. dysenteriae* are as follows. The peripheral chromatin, spoke radii, and karyosome or karyosomal bodies of the two species are respectively similar in location, and are similar, though not identical, in structure. The intermediate zone and the halo are also homologous in location and relations, but differ markedly in the motile phases of the two species (compare figures 20 to 31, with figures 32 to 43, pl. 17).

NUCLEAR STRUCTURE IN *ENDAMOEBA GINGIVALIS* AND IN *E. DYSENTERIAE*

The analysis which we have made of the nuclear structure of *E. gingivalis* reveals variability in dimensions, form, number, and arrangement of the karyosomal bodies, and in the picture of the karyosomal halo according to the degree of decolorization. It will be at once apparent to all familiar with the finer details of nuclear structure in *E. dysenteriae* that this amoeba differs strikingly from the amoeba of the gums in the structure of the karyosome and the region immediately enveloping it.

The intermediate zone of E. gingivalis is relatively free from stainable granules while that of E. dysenteriae is clouded with them. The karyosomal halo of E. gingivalis is composed of a deeply stainable cloud of granules with a darker central sphere around the several karyosomal granules. The halo of E. dysenteriae is free from structures other than spoke radii and the karyosome is single and spherical.

Another measurable difference is that the diameter of the halo of *E. gingivalis* is one-half, or more, of that of the nucleus, while in *E. dysenteriae* it is often only 40 per cent or less. Our figures (pl. 17, figs. 32–43) give a larger proportion than is normal of nuclei with large halos in *E. dysenteriae*. These cytological differences are sufficient to make feasible a clear distinction between infections by these two species in the motile phases, which are the only ones thus far known to occur in tissues.

On the basis of these differences it is clearly evident that the nucleus of *E. gingivalis* is structurally distinct from that of *E. dysenteriae*. This is in addition to the distinctions resting upon their initial areas of infection in the bucco-pharyngeal and intestinal regions respectively, to the encystment of the latter, and to the seeming absence or at least rarity of the process in the former, and to the differential or predominant feeding of one upon leucocytes and of the other, in the motile phases in acute dysentery, upon erythrocytes.

In demonstration of the nuclear conditions in *E. dysenteriae* we have made a detailed structural analysis (pl. 17) of the nuclei of motile amoebas from the bone marrow in arthritis deformans (fig. 32); from the sputum in amoebic abscess in the human lung (fig. 33); from human skin ulcer (fig. 34); from dysenteric ulcer in acute amoebic dysentery in man (figs. 35–36); from the inguinal lymphatic gland in Hodgkin's disease (fig. 37); from human amoebic liver abscess (figs. 38–39); from areas of active ulceration in the colon of the cat induced by amoebas from an active human case (fig. 40); from the stool in chronic human intestinal amoebiasis (figs. 41, 42); and from the stool in acute human intestinal amoebiasis (fig. 43). As stated in the explanation of the plates this material is not the same in freshness at fixation, or in method of fixation. The staining in all cases was done by us and is by a uniform iron-haematoxylin method.

The constant features in all these nuclei of *E. dysenteriae* from these various sources are: the single karyosome, usually spherical but sometimes irregular, and not infrequently excentric; the *clear* halo around it; the granular intermediate zone; and the rather evenly distributed peripheral chromatin.

In contrast with these nuclei, those of *E. gingivalis* have more frequently an *irregular* and often subdivided karyosome, a *granular* halo often of a relatively larger size, with denser periphery and inner sphere, a clear intermediate zone, and more clumping, and less regular distribution of the peripheral chromatin. The outline of the nucleus is more liable to local deformation and it is smaller on the whole than in *E. dysenteriae*.

We have thus far been unable to find a sufficient number of stages of mitosis in *E. gingivalis* to determine the number of chromosomes. It is six in *E. dysenteriae* as will be shown in a paper soon to be published.

The data here presented indicate that *Endamoeba gingivalis*, known thus far only in the motile stage, has a nucleus of different structure from that of *E. dysenteriae* in the motile phase both as it occurs in the bowel and in various tissues. Specifically, the nuclei of amoebas in bone marrow in arthritis deformans are decisively of the type in *E. dysenteriae.* They do not resemble in any particular the nuclei of *E. gingivalis.* We have found no evidence that the amoebas usually associated with gingival abscesses occur in the bone marrow in arthritis deformans of Ely's second type.

On the other hand it is possible that *E. dysenteriae* might occur in abscesses in the buccal region. Attention is directed to the desirability that all cases of such abscesses should be investigated critically to determine the species of amoeba present in the tissues.

SUMMARY

1. The food vacuoles of *Endamoeba gingivalis* contain leucocytes or their nuclei in stages of liquefaction and digestion. Erythrocytes were not found. Certain bacteria also occur but are quantitatively a minor element in the food. In smears the siderophile leucocytic content of the food vacuole is sometimes ejected in a viscous string through the ectoplasm and pellicle.
2. The ectoplasm has a differentially stainable pellicle.
3. The nucleus is proportionally smaller than in *E. dysenteriae,* is less regularly spherical, and more often deformed by pressure.
4. The peripheral chromatin is less regularly distributed than in *E. dysenteriae* and is often clumped in unequal masses irregularly distributed. Some of these masses are connected with the karyosome by spoke radii.
5. The karyosome is generally subcentral, sometimes central, irregular, or angular, often subdivided into two or three or even four granules, while that of *E. dysenteriae* is more generally, but not always spherical, larger, and except at the mitotic period, not subdivided. In the motile phase of the amoeba it is very often excentric.
6. The karyosomal halo in *E. gingivalis* is granular, stains more deeply than the surrounding intermediate zone from which it is separated by a peripheral layer of larger, more deeply staining granules which resist decolorization longer than does the inner part of the halo. Not infrequently it contains a denser sphere around the karyosome. Angular projections are often seen where the spoke radii enter it. In *E. gingivalis* the karyosomal halo is generally, but not always relatively larger than in *E. dysenteriae.* The halo of *E. dysenteriae* is clear and spherical and the layer of demarcation from the intermediate zone is not structurally so clearly differentiated as a distinct darker layer.

7. The intermediate zone in *E. gingivalis* is clear or less stainable than the karyosomal halo while the reverse is true in *E. dysenteriae*.

8. The structure of the nucleus of the motile phase affords a satisfactory and critical basis for separating *E. gingivalis* from *E. dysenteriae* in motile stages. Cysts are unknown as yet in *E. gingivalis*. Reports of their detection are unconvincing.

9. Motile amoebas in human bone marrow have the nuclei of *E. dysenteriae*, not of *E. gingivalis*. Their nuclei are of the same type as in *E. dysenteriae* from the colon of experimentally infected cats, from amoebic ulcers from the colon of man, from human stools in dysentery and in chronic amoebiasis, from human liver abscess, from the sputum in human lung abscess, from amoebic ulcers of human skin, and from the inguinal gland in Hodgkin's disease.

10. The possibility that *E. dysenteriae* rather than *E. gingivalis* was the amoeba present in the malar abscesses reported by Flexner (1892), Kartulis (1893), Verdun and Bruyant (1905*a* and *b*), Dubar and Leroy (1907), Bruyant and Pélissier (1909), and Lynch (1916), and by Artault (1898) in lung abscesses should be held open. Dobell's unsupported conclusion that the amoebas of these abscesses were *E. gingivalis* or endothelial or other cells and not amoebas of any sort must await fuller evidence of the supposed, but not as yet proved, tissue-invasion capabilities of *E. gingivalis*, and a more critical knowledge of the nuclear structure of the amoebas found in such abscesses.

11. We find no evidence of *Endamoeba gingivalis* in bone marrow in arthritis deformans of Ely's second type. The nuclei of the amoebas in the bone marrow are distinctly of the type in *E. dysenteriae* in the motile phase elsewhere in the tissues, or in the bowel. The degree to which *E. gingivalis* invades tissues is still to be determined.

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EXPLANATION OF PLATES

Figures of *Endamoeba gingivalis* (Gros, 1849), Brumpt, 1913, from smears from human gingival exudate fixed in hot Schaudinn's fluid and stained with iron haematoxylin, except where otherwise stated. × 2500 except on plate 17.

PLATE 14

All figures drawn from amoebas in fresh smears in physiological salt solution and iodine-eosin preparations.

Fig. 1. Amoeba that has just been killed in the margin of the iodine-eosin part of the smear. Note the ten pseudopodia thrust out in various directions. The nine earlier-formed ones were stained pink with the eosin and are granular, while the last one thrust out was still colorless.

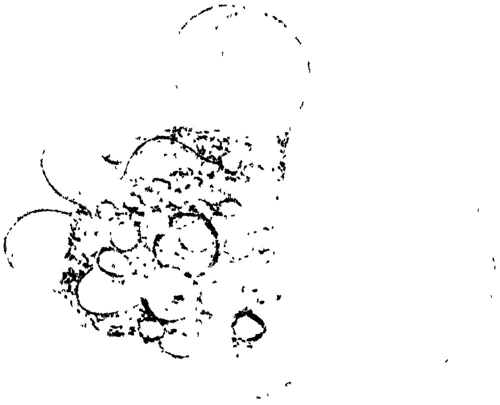
Fig. 2. Active amoeba in physiological salt solution with single pseudopodium thrust out in forward progressive movement. Nucleus cannot be differentiated among food vacuoles.

Fig. 3. Amoeba with single anterior pseudopodium stained with iodine-eosin.

Fig. 4. Active amoeba in physiological salt solution showing typical method of pseudopodial formation when not moving in a forward direction. Nucleus apparent near the center. Few food vacuoles.

Fig. 5. Active amoeba in same material with large pseudopodia at opposite poles.

Fig. 6. Amoeba in active forward progression showing a single hyaline anterior pseudopodium and the posterior tip with its accumulated mass of bacteria and débris on and about adhesive cone.



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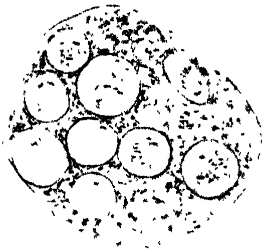

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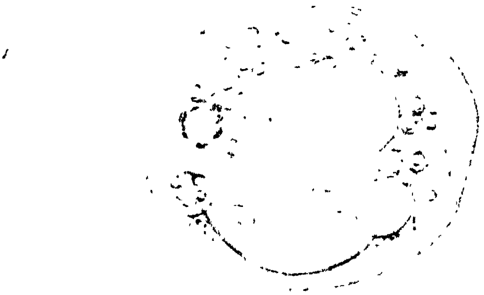

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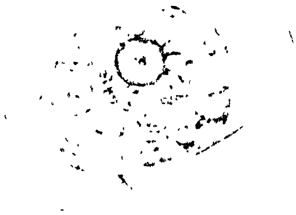

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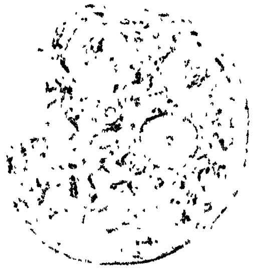

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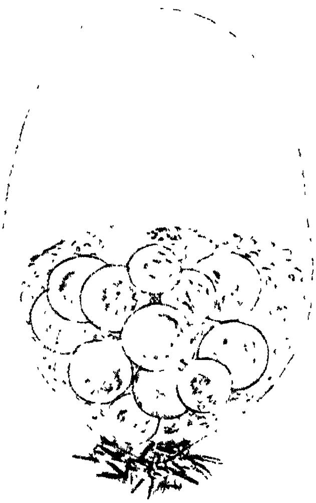

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PLATE 15

Endamoeba gingivalis

Fig. 7. Amoeba ingesting large bacillus, similar to one lying in immediate neighborhood, with the inner end in a food vacuole and already showing action of digestive fluid. Very little chromatin on the nuclear membrane in plane of view. Numerous small rod-shaped bacteria occupy elongated food vacuoles in the cytoplasm. Traces of three small pseudopodia.

Fig. 8. Amoeba containing partly digested nuclei of leucocytes and various rod-shaped bacteria. Remnants of food contents in the smaller vacuoles. No ectoplasm.

Fig. 9. Amoeba with three large food vacuoles with leucocytic nuclei, two of which still show traces of adherent cytoplasm. Small vacuoles with remnants of food bodies but no bacteria.

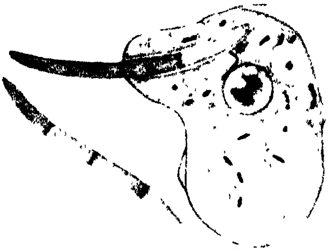
Fig. 10. Binucleate amoeba constricting at the middle as though in the final stage of fission. Nuclei at opposite poles. One large vacuole with a polymorphic leucocytic nucleus. Numerous small vacuoles containing remnants of food bodies. Note irregular distribution of the peripheral chromatin in the nucleus at the right.

Fig. 11. Amoeba with leucocytic food bodies well digested except in one vacuole from which the viscous siderophile substance is being ejected through a canal through the cytoplasm and a ring of deeply stained substance in the pellicle.

Fig. 12. Amoeba with a peripheral film of ectoplasm, but no pseudopodia. Food vacuoles with bacteria and with leucocytic nuclei, the largest one of polymorphic type. Note the sparseness of peripheral chromatin adjacent to the vacuoles.

Fig. 13. Amoeba with retracted pseudopodia, peripheral ectoplasm, three leucocytic food vacuoles.

Fig. 14. Moribund abnormal amoeba, staining very lightly in the midst of a well stained slide. Note the nearly empty food vacuoles, the nearly homogeneous cytoplasm, the ghost of the nucleus which scarcely stains at all, and the disorganized chromatin and karyosome. Spirochaete-like organism in one vacuole.



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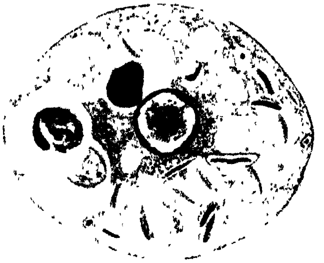

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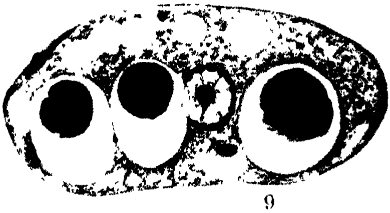

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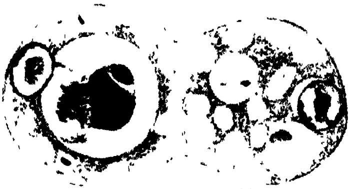

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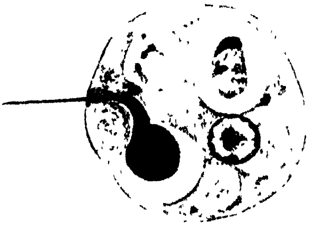

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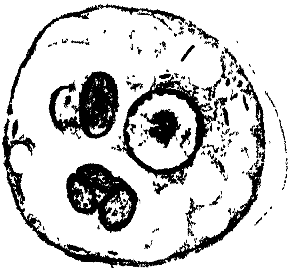

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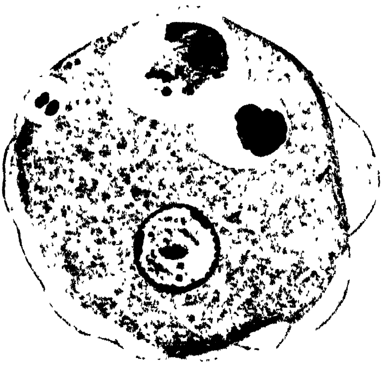

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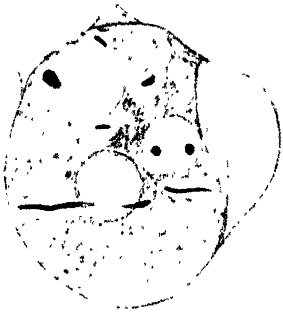

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PLATE 16. **Endamoeba gingivalis**

Fig. 15. Cluster of amoebas on filamentous mass of *Leptothrix*. Note the large numbers of leucocytic food vacuoles. Compare Goodrich and Moseley's (1916, pl. 14, fig. 14) indications of a similar association of these amoebas with *Leptothrix*. × 1200.

Fig. 16. A small amoeba with ectoplasmic pseudopodia at opposite poles, no food bodies but many vacuoles.

Fig. 17. An amoeba as in full swing of locomotion with a single broad pseudopodium, condensed endoplasm and traces of a posterior cone of attachment or holdfast.

Fig. 18. A large amoeba with three pseudopodia, the latest and largest forming anteriorly. Endoplasm crowded with food vacuoles containing leucocytic nuclei. Nucleus compressed, peripheral chromatin reduced to a thin layer.

Fig. 19. An amoeba with numerous small food vacuoles containing bacteria only. Peripheral chromatin in a nearly uniform layer, single karyosome in deeply stained halo. Two pseudopodia.

PLATE 17

All figures magnified 5000 diameters. Nuclei, except that in figure 42, drawn at equatorial focal plane in optical section. The karyosome is also drawn in optical section wherever located.

Endamoeba gingivalis

Figs. 20–31, inclusive. Note smaller size, greater deformation, granular halo, darker inner sphere, subdivided karyosomes, clear intermediate zone, and irregular peripheral chromatin.

Fig. 20. Nucleus not deformed. Note peripheral chromatin on nuclear membrane in interrupted, irregular clumps, very dark border to the granular halo and single, regular karyosome in small dark area. Lightly destained. Amoeba 11 by 14 microns.

Fig. 21. Note irregularity of peripheral chromatin, large angular granular halo, and single karyosome asymmetrical in central darker area. Amoeba 10 by 16 microns.

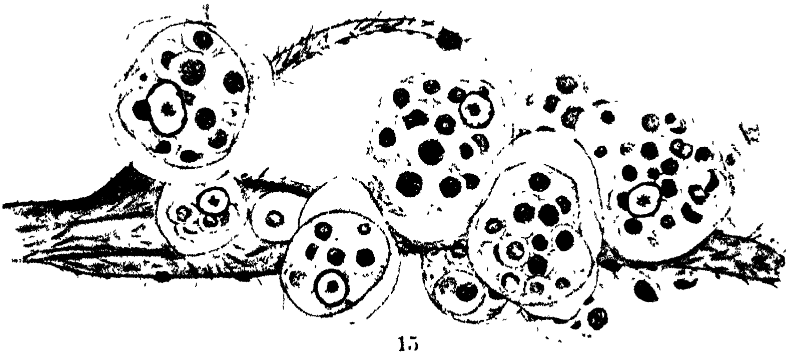
Fig. 22. Peripheral chromatin locally clumped, granular halo more decolorized, one large karyosomal granule excentric in darker area. Amoeba 13 by 14 microns.

Fig. 23. Note deformation in contour, uniform layer of peripheral chromatin, very irregular, deeply stained halo, not decolorized sufficiently to reveal the karyosome. Amoeba 14 by 16 microns.

Fig. 24. Deformed, peripheral chromatin very irregular, heavily clumped, granular halo destained except in the distinct inner sphere immediately around the three subequal karyosomal granules. Granular material encroaching upon the intermediate zone. Outer border of halo more deeply stained. Amoeba 13 by 19 microns.

Fig. 25. Heavily decolorized, deformed, peripheral chromatin thin and clumped, granular halo with marginal rim and darker inner sphere about two karyosomal granules. Intermediate zone invaded by granules on one side. Amoeba 13 by 15 microns.

Fig. 26. Deformed, uniform, beaded peripheral chromatin, partial border to halo, central granular deeply stained mass around two spherical karyosomes. Possibly an early prophase of mitosis. Amoeba 13 by 14 microns.



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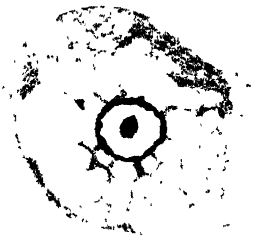

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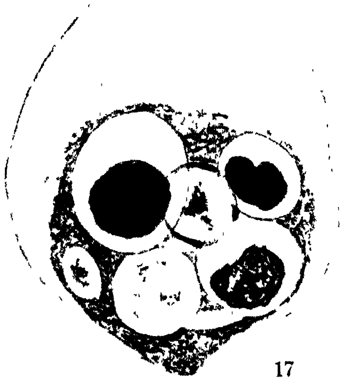

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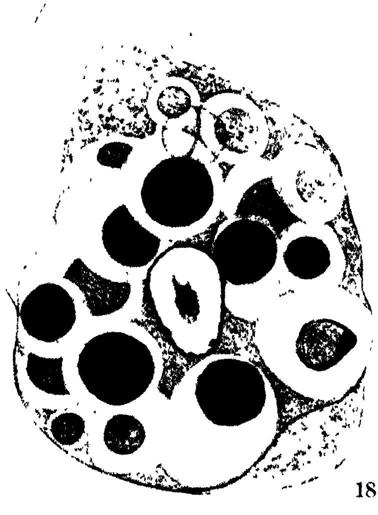

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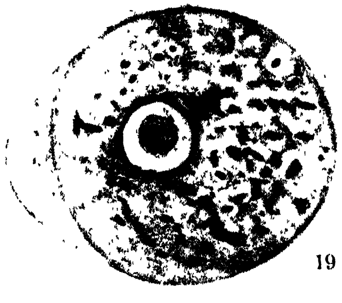

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Fig. 27. Deformed, sharply limited granular halo around three irregular karyosomal granules in darker inner sphere. Peripheral chromatin massed on one side. Amoeba 15 by 19 microns.

Fig. 28. Heavily decolorized, deformed, slightly irregular, beaded peripheral chromatin. Sharply defined granular halo around darker central sphere with three karyosomes. Amoeba 18 by 18 microns.

Fig. 29. Heavily decolorized. Peripheral chromatin massed on one side, granular halo sharply defined, with central inner sphere containing several crowded karyosomal granules. Amoeba 15 by 19 microns.

Fig. 30. Heavily decolorized. Peripheral chromatin uniformly spread or locally clumped, granular halo angular, sharply limited, with an irregular central inner sphere with three karyosomal granules in an asymmetrical position. Amoeba 10 by 14 microns.

Fig. 31. Heavily decolorized, deformed, peripheral chromatin very thin, locally clumped. Granular halo very large, excentric, central inner sphere delimited by a granular zone with a central group of four granules. Possibly an early prophase of mitosis. See Dobell (1919, pl. 5, figs. 93, 94) for somewhat similar halo. Amoeba 17 by 19 microns.

Endamoeba dysenteriae

Figs. 32–43 in motile phases. Note larger size, more uniformly distributed peripheral chromatin, granular intermediate zone, clear spherical halo, and single karyosome, centrally located in the halo but often excentric in the nucleus.

Fig. 32. From bone marrow of head of femur in arthritis deformans. Fixed in formalin, decalcified, refixed in Schaudinn's fluid. Peripheral chromatin small in amount, clumped locally. Amoeba 8 by 12 microns.

Fig. 33. From cold sputum from pulmonary amoebic abscess. Peripheral chromatin regularly distributed and locally clumped. Amoeba 19 by 27 microns.

Fig. 34. From amoebic ulcer of skin in fatal case of amoebic liver abscess, fixed three days after death. Chromatin uniformly layered on the membrane. Amoeba 18 by 27 microns.

Figs. 35 and 36. From autopsy material of amoebic ulcer of colon from amoebas in tissues. Peripheral chromatin uniformly distributed, beaded or layered. Amoebas 17 by 21 and 16 by 19 microns, respectively.

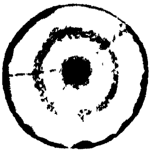
Fig. 37. From inguinal lymphatic gland in Hodgkin's disease removed by operation and fixed in formalin. Peripheral chromatin layered. Amoeba 17 by 18 microns.

Figs. 38, 39. Autopsy material from liver abscess. Peripheral chromatin beaded and somewhat irregular. Amoebas 12 by 15 and 16 by 21 microns, respectively.

Fig. 40. From purulent surface of colon of cat infected from an acute case of human amoebiasis. Peripheral chromatin very irregularly clumped. Amoeba 23 by 28 microns.

Figs. 41 and 42. From amoebas in stool of chronic human amoebiasis. Peripheral chromatin rather uniformly distributed with local clumping in rather uniform blobs. Fig. 42. Nucleus viewed in contour. Amoebas 11 by 11 and 8 by 11 microns, respectively.

Fig. 43. From foecal smear from a case of acute amoebic dysentery. Chromatin uniformly clumped. Halo very large with aggregations of stainable granules at junctions of spoke radii. Amoeba 22 by 22 microns.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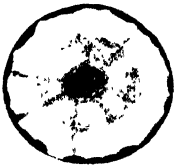
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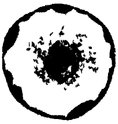
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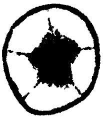
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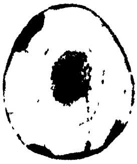
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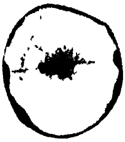
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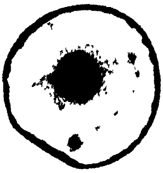
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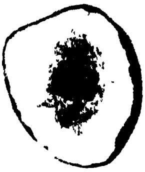
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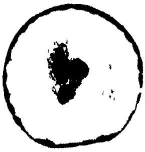
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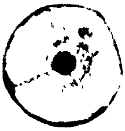
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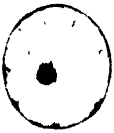
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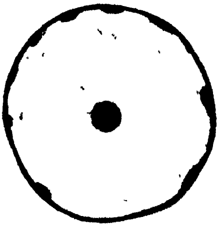
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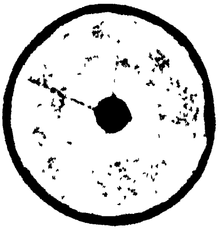
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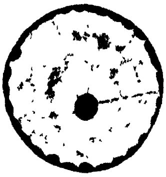
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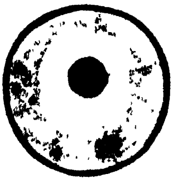
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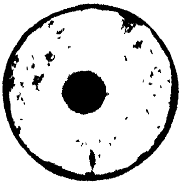
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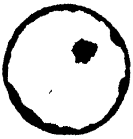
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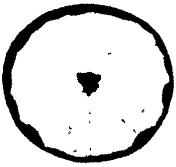
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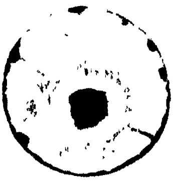
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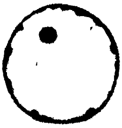
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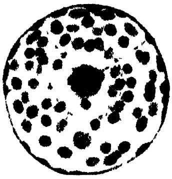
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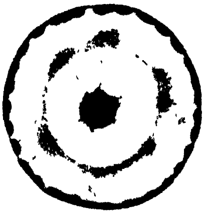
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PLATE 18

Endamoeba gingivalis

× 2500

Fig. 44. Two amoebas joined by fusion of ejected siderophile strands from leucocytic food vacuoles. Note siderophile rings in the pellicle at orifices of emergence. Food vacuoles with leucocytic contents only. No ectoplasm visible.

Fig. 45. A similar pair of amoebas whose juxtaposition suggests retraction of the viscous siderophile substance of the two vacuoles which had fused. No ectoplasm visible.

Fig. 46. An amoeba whose food vacuoles have no formed contents. A siderophile thread appears to have been extruded through the cytoplasm and to have rococheted five times across the endoplasmic mass, rebounding at or near its periphery, with one end emerging through the ectoplasm. The vacuole from which it originates lies near the siderophile ring from which a protoplasmic process has been extruded. Ectoplasmic zone sharply delimited.

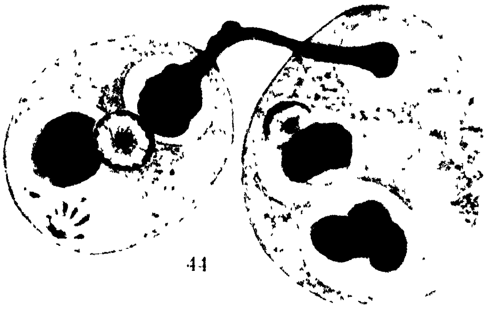
Fig. 47. Apparently moribund. Ectoplasm and endoplasm not clearly delimited. Chromatin deliquescent on membrane. Food vacuoles empty or abnormal.

Fig. 48. Small amoeba with no ectoplasm visible. Coiled elongated bacterium in food vacuole.

Fig. 49. Large amoeba crowded with food vacuoles exclusively with leucocytic contents. Deeply stained granular halo on the nucleus. Siderophile spheres in food vacuoles with concentric peripheral laminations. One vacuole extruding a siderophile thread.

Fig. 50. Siderophile substance emerging from three lobes of a leucocytic food body in three strands and fusing peripherally.

Fig. 51. An amoeba with no ectoplasm visible, crowded with elongated food vacuoles each with an elongated rod-like bacillus.



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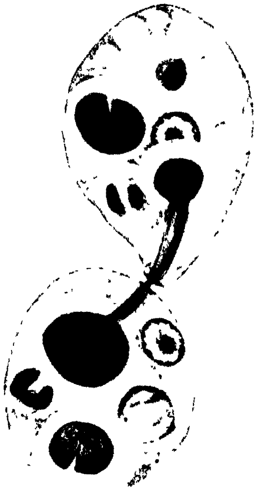

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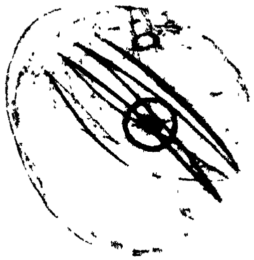

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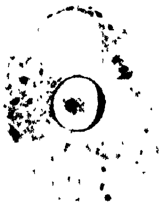

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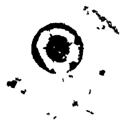

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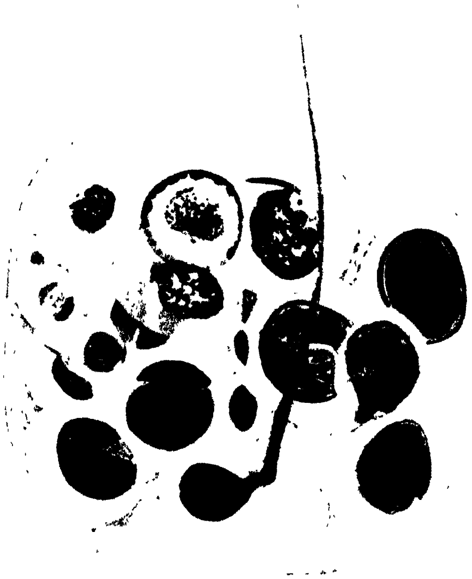

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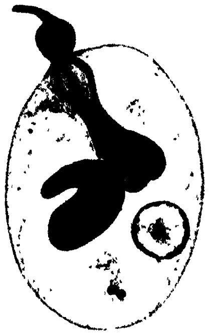

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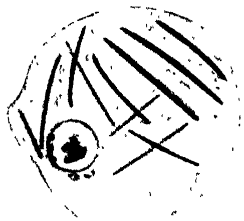

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MORPHOLOGY AND MITOSIS OF DINENYMPHA FIMBRIATA SP. NOV.

BY

HAROLD KIRBY, Jr.

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MORPHOLOGY AND MITOSIS OF DINENYMPHA FIMBRIATA SP. NOV.

BY

HAROLD KIRBY JR.

This paper describes the structure, nuclear division, and binary fission of a species of *Dinenympha*, a flagellate from the intestine of the termite *Reticulotermes hesperus* Banks found in Berkeley, California. A fairly complete series of mitotic figures was found in my preparations, affording an opportunity for a more complete description than has previously appeared.

I am indebted to Dr. Olive Swezy for assistance in the technique of preparing the plates and for advice in the preparation of the manuscript; and to Dr. C. A. Kofoid for the facilities and counsel which made the work possible, for criticism and interpretation, and for access to unpublished work. A comparison with the flagellates of the eastern *Reticulotermes hesperus* Kollar, the species on which Leidy (1881) and Porter (1897) worked, was made possible by the kindness of Dr. L. R. Cleveland in sending me material from Baltimore.

Material and technique.—*Reticulotermes hesperus* is commonly found under the bark of dead pine stumps in the Berkeley hills back of the University of California campus. The organisms here described constitute one of the more abundant species of the seething mass of Protozoa found in the hind gut of this termite.

Studies of living material were made by diluting the contents of the hind gut with Locke's solution or with normal saline. In either of these solutions the animals die after an hour or less, but can be observed for a while in active movement. For killing and fixation, hot Schaudinn's fluid was used. The preparations were stained with Heidenhain's iron alum haematoxylin and were mounted in balsam.

Most of the studies recorded in this paper deal with one series of slides made on March 1, 1923; and with one slide in particular upon which occurred hundreds of division stages of both *Dinenympha* and *Pyrsonympha*. *Pyrsonympha* was in division on several slides of this series, so that comparative material was available. *Dinenympha fimbriata* has been found also on other slides and other series from different colonies of termites, but occurrence of division stages was rare.

GENERAL MORPHOLOGY

Dinenympha fimbriata appears in abundance in *Reticulotermes hesperus,* associated with other species of *Dinenympha* and other flagellates. A flagellate of very similar appearance appears in *R. flavipes,* but there is sufficient difference for specific distinction. It is much less abundant than *D. fimbriata.* It is this flagellate, evidently, that Porter (1897) and Leidy (1881) have described as a young form of *Pyrsonympha* occurring in *R. flavipes.* Together with *Pyrsonympha,* it appears in *R. hesperus,* both free in the lumen and lining the wall of the gut; in the latter location, it is long and narrow and filled with wood particles. It is covered with filamentous appendages, which are supposedly bacteria, and it is because of this fringe of bacteria that the name *D. fimbriata* is applied.

Two forms are present. One of these (pl. 19, figs. 4–7) is rather broad and thick, twisted or turned once, and does not show distinct edges. The figures on the plate represent the range in size; the smallest 52 microns long, the largest 78. The average size is about 64 microns long by 18 microns wide at the middle of the body. There are eight flagellar cords, which run from the centroblepharoplast to become free at the posterior end of the body. This point, however, is hard to determine because of the large number of bacteria, which form a thick caudal tuft. In living material the movement of the flagella may be observed as distinct from that of the bacteria. There is an axostyle present, running under the pellicle to become attached at the posterior end of the body, generally between the third and fourth cords from one edge. It varies in thickness, but is uniform throughout its length. In some (pl. 19, fig. 6) it is very fine; in others (pl. 19, fig. 4) it is heavy.

The general body form resembles that of *Pyrsonympha,* but the cords are limited to the out-curved body surface and are not distributed over the whole surface as in that genus. The body is usually club-shaped, and is bent somewhat into an S-shaped curve. There is little locomotion, the animal remaining in one place, while the whole body undulates. The interior is filled with wood particles.

There are numerous filaments all over the surface, with usually an especially thick tuft of these at the posterior end. These filaments appear to be equally numerous over the rest of the surface, both where the cords are present and where they are not. Sometimes these filaments appear to be straight, sometimes wavy; they project from the surface in different directions, stain deeply, and are of uniform thickness to the tip. The ends of some, perhaps, are on the flagellar cords, but those of others certainly are not; and the distribution is by no means regular. No granule could be observed at the point of attachment of any of these. Observations on living material and a comparison with flagella such as those of *Holomastigotes* and *Trichonympha* lead to the conclusion that these are adherent bacteria, as suggested by Grassi in 1893. They are spiraled much like the smaller spirilla, and are of the same form as the unattached bacteria. There is no doubt that bacteria may become attached; their presence in bunches on otherwise bare protozoa makes that evident. On one occasion some were observed to swim actively up to a *Dinenympha* and adhere by one end; and others were seen to break loose and swim away.

Janicki (1915, p. 590) states that bacterial filaments are often found attached to the body of *Stephanonympha.* These vibrate in such a way that the body appears to be covered with flagella. He describes (1915, p. 591) bristle-like flagella on the posterior part of the body of *Parajoenia,* each of which begins close to a rounded body. It is not unlikely that these "bristle-like flagella" may have been attached bacteria. Grassi, it has been noted, described the filaments clothing the body of some *Dinenympha* individuals as attached spirilla. Comes (1910) figures numerous "flagelliform spirillae" on the body of *Lophophora vacuolata,* which is a *Pyrsonympha.* De Zulueta (1915) does not commit himself, but speaks of the filiform appendages which correspond to the attached bacteria or flagella of other authors. Koidzumi (1921) writes of them as "filamentous appendages," some of which, he says, are probably attached microorganisms, others homologous with the bristles that Janicki found on *Parajoenia.* The arrangement of these structures on transverse rows such as he described for his type 4 of *D. porteri* has not been observed in *D. fimbriata.* Evidently the attachment of bacteria to the body surface of these protozoa is common.

The other form of *Dinenympha fimbriata* is elongate, cigar-shaped, and constant in form (pl. 19, figs. 1–3). It ranges in length from 42 to 65 microns, in width from 6 to 10 microns. The average size is about 52 microns long and 8 wide. There are four flagellar cords, and the body is wound slightly into a laeotropic spiral and more distinctly edged than it is in the first type. The posterior end is rounded, with a tuft of bacteria attached. The axostyle is a broad band which tapers abruptly at the tip, where it is attached to the body wall. In some cases (pl. 19, fig. 1) the attachment does not appear.

These two forms are probably developmental stages of the same species. The form with four flagellar cords is one that has recently divided and has not yet doubled this number. The axostyle is a broad band, and might be of a substance that decreases in amount through contribution to growth until it becomes narrow as in the first form. Its failure to attach sometimes may be due to its being in an earlier stage, for it grows back from the basal granule. Division was found only in the eight-corded forms. In these large forms axostyles of intermediate thickness sometimes appear (pl. 19, fig. 4), and suggest the plausibility of the interpretation given above. This phenomenon may be compared to the absorption of the substance in the parabasal and chromatoidal bodies during growth and activity.

The deeply staining bodies shown filling the body in plate 19, figure 7, were seen in two specimens of the eight-corded form on the same slide. The endoplasm contained an abundance of woody particles, and in it were about 40 groups of the organisms under consideration. In shape, these were stout, curved rods; in size, one to two microns long. Many of them were in pairs, but some were in groups of three, four, five, and as many as seven. Some of them were to be seen on end, when they appeared as compact bundles of rods. They stained very deeply with iron haematoxylin. Probably they were intracellular bacteria.

NUCLEAR DIVISION

The resting nucleus.—The pyriform nucleus is situated in the anterior part of the body and we have never seen it removed from this position, as it is said to be sometimes in *Pyrsonympha,* and as it is in *Dinenympha rugosa* according to Koidzumi. There is a centroblepharoplast to which the nucleus is attached by a rhizoplast, and from which the flagella and axostyle arise. These details are difficult to make out in the full-grown *Dinenympha,* but in recently divided forms they appear distinctly. There is a discrete nuclear membrane. In the clear karyolymph is imbedded the linin reticulum, at the nodal points of which are the few chromatin granules, widely scattered and small. In the nucleus, usually in the posterior part, is a spherical body, one to two microns in diameter, and the endosome, which stains deeply with iron haematoxylin. The peripheral chromatin stains lightly, so that this body stands out very clearly (pl. 19, figs. 4–6). Often the endosome, or nucleolus as it may be called from homology with the nucleolus elsewhere, appears vacuolated internally. Usually there is only one of these, but sometimes there are two or three smaller ones. The structure of the resting nucleus recalls that of certain Sporozoa and Rhizopoda.

In the *prophase* the nucleolus does not break up, as in *Pyrsonympha,* but becomes paler and smaller and drawn out into points which continue into the linin reticulum (pl. 22, figs. 24, 30). The approach of nuclear division is heralded by the appearance of more numerous and larger chromatic granules. The reticulum breaks down, and the fibers are gathered into threads on which chromatin becomes incrusted. Then, instead of a plexus of linin fibers with rather small meshes, there is a sinuous arrangement of black (with haematoxylin) and somewhat knotty threads which are connected by unstained portions of the linin reticulum where chromatin has not condensed. At this time the nucleolus is undergoing decrease in size, and eventually it disappears.

There is not a continuous spireme. The process of condensation continues, and the chromatin threads shorten until they become elongate chromosome-like structures still connected by achromatic fibers. At this stage, where the chromosomes are simple threads, splitting seems to occur. In the nucleus shown in plate 19, figure 4, many of the chromosomes seem to be doubled by a longitudinal split: in some this is unmistakable. Later on the split is obscure, so that pairing of chromosomes could not be determined in stages on the equatorial plate. Thus the longitudinal splitting seems to take place long before the organization of the spindle. The chromosomes then shorten and thicken and produce the V-shaped, looped structures of plate 22, figure 39. The number is between 25 and 30.

The centroblepharoplast divides, and a paradesmose is drawn out between the two parts. The flagellar cords separate into two groups of four each, each group connecting with a granule at one of the poles. The axostyle, meanwhile, has lost its connection and is cast into the endoplasm, where it soon loses its staining capacity and is resorbed (pl. 20, fig. 10). In the later prophase stages it has entirely disappeared and new ones have begun to grow at the two poles, each connected by a strand to the granule there.

During the prophase the body rounds up and the flagellar cords become disposed over the surface without restriction to one side. The shape is irregular and amoeboid; in most cases the body contains bits of wood and retains its fringe of bacteria. The presence of wood particles and bacteria in these division stages, as well as size, method of division, and other points, was used as a criterion to distinguish between *Dinenympha* and *Pyrsonympha.* Probably some dividing *Dinenympha* have neither food bodies nor adherent bacteria, but in order to avoid error all figures except nos. 20 and 21, plate 21, were drawn from bacteria-clothed forms.

The endoplasm at this time is decidedly aveolar, and there are many chromatic granules between the alveoles. The flagellar cords are wavy and irregular.

In several instances a contraction phase of the chromatin material in the nucleus was observed. Of such nuclei three are figured (pl. 22, figs. 36–38) and more were seen. That these are prophase and not telophase stages is evidenced by the absence of the axostyle and the presence of eight cords. At one side of the nucleus the chromatin is contracted into a deeply staining mass, and in one case (pl. 22, fig. 37) a body resembling the nucleolus was free of this mass. In another case the chromosomes were loosened up and some were manifestly V-shaped. This phenomenon resembles synizesis, but the significance here is obscure.

In the late prophase intranuclear spindle fibers appear. These fibers stain heavily with iron haematoxylin and apparently are attached to the chromosomes. Stages are to be seen where they do not yet form a spindle, as they diverge from granules that have not yet taken up a position at the poles (pl. 22, fig. 41). The nuclear membrane remains intact through all this process, and the achromatic figure occupies only a part of the nucleus.

In the *metaphase* (pl. 20, figs. 11–14; pl. 22, figs. 42–44) the chromosomes are arranged in a mass on the equatorial plate. Probably, although this point could not be determined, the halves of the double chromosomes separate and migrate along the lines of the spindle fibers, leaving between the two groups the alveolar nucleoplasm and the fibers.

At the poles of this very distinct spindle, sometimes slightly removed from the membrane, are distinct black granules. In some cases each granule appears to be attached by a thread to a small granule on the nuclear membrane. For this reason the granule in the early stages before it has divided is called the centroblepharoplast; later, after the paradesmose is drawn out, it divides into centrosome and blepharoplast, as in *Trichomonas* (Kofoid and Swezy, 1915). The incipient axostyles, very small at this time, are attached to the blepharoplast by relatively long threads. By the onset of the anaphase (pl. 20, fig. 15; pl. 21, figs. 16–22; pl. 22, figs. 45–48) these axostyles have grown large, being at least as long as the nucleus.

The chromosomes, which have separated in the metaphase and migrated to the poles, form dense masses there. Some fibers appear between these groups of chromosomes, and in one instance (pl. 21, fig. 16) a line which was interpreted as the remnant of the paradesmose was seen to lie over the nuclear membrane.

The nucleus constricts and divides; the membrane draws out into long points. After this separation takes places much of the wood seems to be extruded from the body, for at this time and later few wood particles are present. In most cases the adherent bacteria are present throughout the process.

Several figures of the anaphase and telophase have shown two connected granules at one pole (pl. 21, figs. 17, 19). To one of these, one flagellar cord, the axostyle, and a rhizoplast from the centrosome are attached; to the other are attached three flagellar cords. This situation suggests that of *Pentatrichomonas*, where there is a primary and secondary blepharoplast (Kofoid and Swezy, 1923).

After the nuclei have separated, division of the body occurs. This takes place rapidly, hence no stages of plasmotomy were seen. The telophase after plasmotomy is distinguished from the prophase by the size, the presence of an axostyle, the four instead of eight flagellar cords, the shape of the body, and the absence of wood particles.

The chromatin is at first collected into a mass at the pole; then the chromosomes scatter until they are distributed throughout the nucleus. Some of them are V-shaped and hooked as in the prophase (pl. 22, fig. 47). There is no nucleolus evident at first, but some globular masses appear and collect to form this body. The chromosomes resolve into threads, these break up into granules, and the condition of the resting nucleus is restored.

DISCUSSION

The endosome.—The word endosome is used for the chromatic mass in the nucleus; its use does not indicate the precise nature of the body. The German term *Binnenkörper* (Doflein, 1916, p. 21) has the same meaning. Vesicular nuclei, such as those of flagellates, rhizopods, and Sporozoa, usually contain such a body. In some cases this is a karyosome, containing chromatin material as well as plastin. A karyosome which divides as the nucleus divides and is distributed to the two daughter nuclei is found in Euglenoidina (*Menoidium*, Hall, 1923; *Euglena, Heteronema*) and protomonads (*Trypanosoma, Bodo*). Hall (1923) uses the term endosome for a body of this nature in the euglenoid flagellate *Menoidium incurvum.* In the higher groups of the Polymastigina the endosome is not the same structure, for it disappears during division and the chromosomes are organized from the peripheral chromatin (*Calonympha,* Janicki, 1915, p. 627). In the Hypermastigina the *Binnenkörper* or endosome likewise disappears, as in *Parajoenia* (Janicki, 1915, p. 593). In certain Phytomonadina there is a similar structure. A nucleolus which disappears during division before the formation of the spindle was found by Hartmann (1918, p. 20) in *Chlorogonium,* and was reported in *Polytoma uvella* by Entz (1918, p. 20). Nucleoli are present in gregarines and other Sporozoa, according to Montgomery (1899) and other authors. The endosome of *Dinenympha* appears to be homologous with this nucleolus of phytomonads, gregarines, higher flagellates, and metazoan cells. Its fate in division is similar and it has a similar structure when at rest. This conception agrees with that of De Zulueta (1915) and Koidzumi (1921), although Koidzumi speaks of it as a karyosome (in *Pyrsonympha*). The staining reactions support this contention, as there is a differential staining capacity such as that of the nucleolus and chromatin material of metazoan cells (De Zulueta, 1915).

The flagellar cords.—The four or eight cords which run backward on the surface of the body and continue as free flagella in the Dinenymphidae are interesting, for the structures as such are peculiar to this group. Leidy (1881, p. 17) writes of these as "undulating lines." Porter (1897, p. 61) refers to them as contractile or muscular cords; but he did not observe the free ends which are to be found in the organisms he described. Grassi (1893, p. 128) calls them *mem-*

branelle ondulanti, similar to those of *Trichomonas.* Comes (1910, p. 6) and De Zulueta (1915) both call them undulating membranes. Koidzumi (1921) uses the term flagellar cords, which term is retained in this paper. It clearly indicates their flagellar origin, yet distinguishes them from free flagella and undulating membranes; for in some species of *Dinenympha* they certainly do not form undulating membranes. They are raised on rather high ridges in some forms, where they appear very much like undulating membranes.

Grassi (1911, p. 730) states that in the family Dinenymphidae the flagella are all turned backward and fused with the surface of the body in the form of undulating lamellae, becoming free at the posterior extremity. Doflein (1916, p. 605) distinguished the family in the same way: *Formen deren 4 Geisseln alle rückwärts gerichtete, mit der Pellicula verschmolzen, undulierende Membranen bilden.* Kofoid and Swezy (1923), outlining the methods of evolution of polymastigote flagellates, state that there are two methods: increase in the number of flagella, and differentiation and specialization of the flagella already present. The flagellar cords of the Dinenymphidae are an extreme specialization of flagella.

Four is the fundamental number of flagellar cords, just as four is the fundamental number of flagella in the Tetramitidae and Calonymphidae; eight is a duplication of the number after one division and prior to the next. Duplication is probably by outgrowth, as is usual in flagellates.

The axostyle.—This structure is a band or rod connected with the centroblepharoplast and extending in *Dinenympha* under the pellicle to end in attachment to the posterior body wall. In *Pyrsonympha* its nature is a little different, as it is free in the endoplasm distally and is capable of vigorous undulations; it may also be frayed out into a number of filaments. At division it is always resorbed and two new ones grow out from the blepharoplasts.

Leidy (1881, p. 16) refers to this structure in *Pyrsonympha* as an undulating cord, but does not mention its occurrence in *Dinenympha.* Porter (1897, p. 62) speaks of it as a flagellum in *Pyrsonympha.* Grassi (1893, p. 128) calls it *bastoncello,* which Blandford (1897) translates "rod." According to Comes it is a *bastoncello scheletrico* (1910, p. 5). Grassi (1911, p. 735) says that he is doubtful of its homology with the axostyle, a point on which Janicki (1915) and Koidzumi (1921) agree with him. Koidzumi (1921) uses the term "axial filament." De Zulueta (1915, p. 10) describes it as an *axostilo* in *Dinenympha.*

The axostyles of many flagellates are hyaline or stain weakly. In certain of the higher polymastigotes, however, as *Stephanonympha, Calonympha,* and *Giardia,* and in the hypermastigote *Lophomonas,* the axostyle stains with iron haematoxylin.

Three methods of origin have been described for the axostyle. Many of the investigators working on polymastigotes have believed that the axostyle arises by the persistence of the paradesmose, as it does in *Lophomonas* according to Janicki (1910). But in most cases there is not enough evidence to support this idea, as Kofoid (1915) brings out. Splitting has been described as the method of duplication in some instances, as in *Trichomonas* (Kofoid and Swezy, 1915). In many others the axostyle disappears and a new one grows out from the basal granules. This last method of origin seems best supported, and, if we are to seek for a common method of origin, the existence of which is not certain, this is the most promising. The evidence is conclusive in *Dinenympha fimbriata* that the new axostyles arise by outgrowth from the blepharoplast.

Janicki (1915, p. 614) states that the axostyle of *Oxymonas* may be homologous with Grassi's *benderella subassile contrattile* in *Dinenympha.* He supposes this structure in *Oxymonas* to arise by persistence of the paradesmose, but from his figure it seems to be possible that origin is really by outgrowth. That these are homologous structures seems likely.

It has been difficult to understand the homologies of this structure. Grassi has given no reasons for saying (1911, p. 735) that the homology with the axostyle is doubtful. Koidzumi (1921, p. 300) says that in his opinion "the axostyle of trichomonad flagellates is not homologous with the axial filament of *Pyrsonympha.* The axial filament is not simply a skeletal organ like the axostyle, but an organ playing the rôles of several different structures in the trichomonads, viz., the axostyle and the basal granule." It has been shown that the axostyle is not merely skeletal, but appears in full motor activity at times, as in *Trichomonas augusta* (Kofoid and Swezy, 1915, p. 311). It is "an intracytoplasmic flagellum highly specialized for motor activity" in *Trichomonas,* according to these investigators. So it is not incompatible with the conception of it as an axostyle that in *Pyrsonympha* it is not merely skeletal but possesses the capacity of motor activity. The conception of Koidzumi that it functions as a blepharoplast for the flagellar cords, or supplies the bodies playing the part of division centers, is rather bizarre. In *Dinenympha* *fimbriata* there is certainly a basal granule to which all neuromotor structures are attached, and a careful investigation of *Pyrsonympha* would doubtless show this to be the case there (see Comes, 1912, p. 290 and pl. 11, fig. 20).

It is of interest to consider the granules to which these neuromotor structures are attached. In *Dinenympha fimbriata* it has been shown that there is a primary and secondary blepharoplast. The axostyle and one flagellar cord are attached to the primary, three cords to the secondary. In *Pentatrichomonas* (Kofoid and Swezy, 1923, p. 378) there is also both a primary and a secondary blepharoplast; the axostyle and parabasal are attached to the primary. When there are two blepharoplasts the axostyle appears to be attached generally to the primary, the main group of flagella to the secondary. In *Chilomastix* (Kofoid and Swezy, 1920) there are three blepharoplasts, and the axostyle (parastyle) arises from the secondary.

The centroblepharoplast.—The term centroblepharoplast is applied by Kofoid and Swezy (1915, p. 26) to the structure consisting of a centriole and the blepharoplast within which it lies and from which it emerges at mitosis. *Trichomonas augusta* has a centroblepharoplast of this sort; the centrosome emerges at division and a paradesmose appears between the divided blepharoplasts. The centrosome remains on the nucleus; the blepharoplast with the flagella attached moves a short distance away, remaining connected to the centrosome by a rhizoplast. In *Trichomitus termitis* the procedure is closely similar but of a somewhat different type, for the paradesmose appears between the centrosomes instead of between the blepharoplasts. In the resting stage there is a centroblepharoplast with which flagella and parabasal connect and which, in division, gives out the centrosome. In *Ditrichomonas termitis* (Cutler, 1919) the blepharoplast divides; then one divides again, giving out the centriole, which migrates toward the nuclear membrane; then the centriole divides, forming the paradesmose, and a connection is established between the second centriole and the other blepharoplast.

In *Dinenympha fimbriata* the process is evidently as in *Trichomonas;* the centroblepharoplast contains the centrosome, which at the time of division emerges, and a paradesmose is established between the blepharoplasts. A secondary division of the blepharoplast then occurs, so that there are two basal granules, with one of which three flagella connect and with the other one flagellum, the axostyle, and the centrosome-rhizoplast. In *Pentatrichomonas*, as has been seen, there are two blepharoplasts: to the primary, the centrosome-rhizoplast, the parabasal body, the axostyle, the independent flagellum and the marginal filament of the undulating membrane are attached; from the secondary the group of four flagella arises. In the resting stages of *Dinenympha* the separation of the granules could not be seen, but in a telophase after division had taken place (pl. 21, fig. 17) the condition was very much as in *Pentatrichomonas.*

The paradesmose.—Many authors have observed in the division of the Polymastigina and Hypermastigina a rod-like structure between the divided blepharoplasts or centrosomes on the outside of the nuclear membrane. Kofoid and Swezy (1915 *a*) proposed the name paradesmose for the chromatic thread connecting the daughter blepharoplasts in the mitosis of *Trichomonas augusta*, and stated that, after the granules part, it "connects the basal granules, and not the centrosomes." Thus, as Hall (1923) brings out, there are two types of paradesmose, and it is convenient to distinguish between a blepharoplast-desmose and a centrosome-desmose. The paradesmose of *Dinenympha* is a blepharoplast-desmose similar to that of *Trichomonas,* since it connects the blepharoplasts after the centrosomes have separated from them.

Dinenympha fimbriata sp. nov.

Form 1.—Body elongate, spindle-shaped, rounded at both ends; flattened and turned slightly into a laeotropic spiral of not more than one turn. Nucleus pyriform, situated at the anterior end of the body, with a nucleolus, peripheral chromatin, and a linin network. Axostyle a broad band, not always attached posteriorly. The endoplasm is filled with wood particles. The surface of the body is covered with adherent bacteria, which form an especially thick tuft at the posterior end. There are four flagellar cords, which become free posteriorly. The length averages 52 microns, the width 8 microns.

Form 2.—The number of flagellar cords has increased to eight. The body has become broader, stouter, and thicker, and is not so distinctly edged. The average size is 64 microns long by 18 microns wide. The body is twisted or turned not more than once. The axostyle is distinct, but not so broad as in form 1. It is a rod rather than a band, and is always attached posteriorly. The endoplasm is filled with wood particles and the surface is covered with bacteria. Mitotic division is of the trichomonad type.

An intestinal flagellate of *Reticulotermes hesperus* Banks from Berkeley, California.

SUMMARY AND CONCLUSIONS

1. *Dinenympha fimbriata* occurs as an intestinal flagellate in the termite *Reticulotermes hesperus* Banks, and is found either free in the lumen or attached to the epithelium.

2. The newly divided form has a stout axostyle and four flagellar cords; as it increases in age eight cords develop, probably by outgrowth, and the axostyle becomes more slender.

3. The flagella are fused with the body surface, beginning at the centroblepharoplast and running backward to continue as free flagella, as in the case of the flagellar border of the undulating membrane of *Trichomonas.*

4. There is a true nucleolus in the nucleus, which disappears in division, as does a homologous body in the Phytomonadina, higher Polymastigina, Hypermastigina, Sporozoa, and Metazoa. The peripheral chromatin is arranged on a linin reticulum and is lightly staining in the resting stage.

5. In the formation of chromosomes the granules collect and concentrate into threads; there is no continuous spireme.

6. The chromosomes multiply by a longitudinal split in the prophase, and separate when on the spindle.

7. The axostyle is resorbed during the division process, and new ones grow out from the blepharoplasts.

8. The eight cords are divided into two equal groups in division, and one group remains attached to each blepharoplast.

9. An achromatic spindle forms within the nuclear membrane, which remains intact throughout the division process.

10. The type of division closely resembles that of trichomonads. There is a blepharoplast-desmose, a separation of the centrosome from the centroblepharoplast, and a division of the blepharoplast into primary and secondary, as is the condition in *Pentatrichomonas.*

11. *Dinenympha* is allied in many respects to the trichomonad group. There has been a specialization of flagella, all having fused with the body surface. Its classification in the Polymastigina close to the Tetramitidae is correct; it is not necessary, as Koidzumi supposed it to be, to found a new order to contain the group.

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EXPLANATION OF PLATES

All figures are of *Dinenympha fimbriata* sp. nov. × 2000, unless otherwise stated. Material fixed in hot Schaudinn's fluid and stained in iron haematoxylin. Drawings made with Abbe camera lucida.

PLATE 19

Vegetative forms of *Dinenympha fimbriata*. × 1250.

Fig. 1. Individual recently divided. The chromatin has not yet resolved itself into the resting condition. The axostyle does not reach the end. Four flagellar cords are present.

Fig. 2. A typical four-corded form. Axostyle attached.

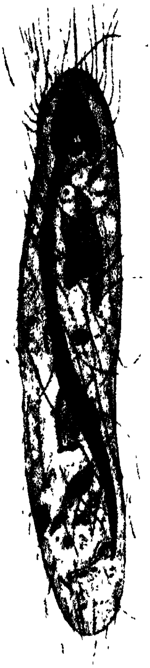
Fig. 3. A four-corded form. Stout, band-like axostyle.

Fig. 4. An eight-corded form with stout axostyle.

Fig. 5. A form of smaller size with more slender axostyle.

Fig. 6. A typical form with eight cords. The axostyle is between cords 3 and 4, and a line of short rods between 5 and 6, counting from the right.

Fig. 7. A large form, nucleus organizing for prophase. An abundance of bacterial parasites present in the endoplasm.



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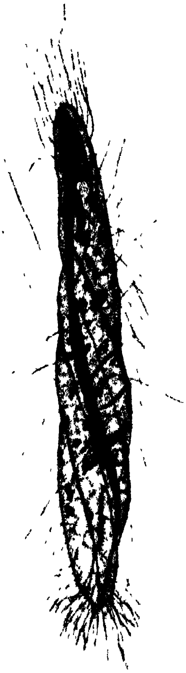

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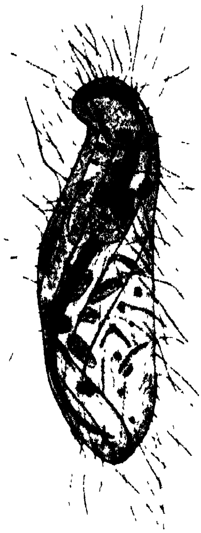

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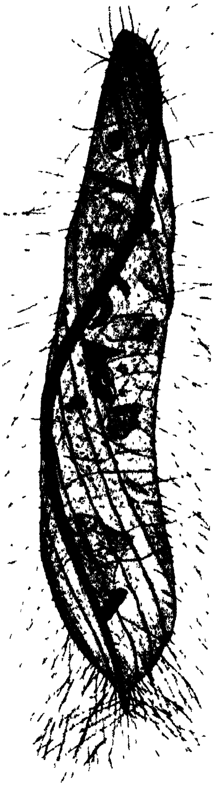

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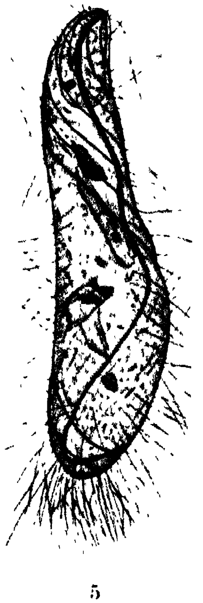

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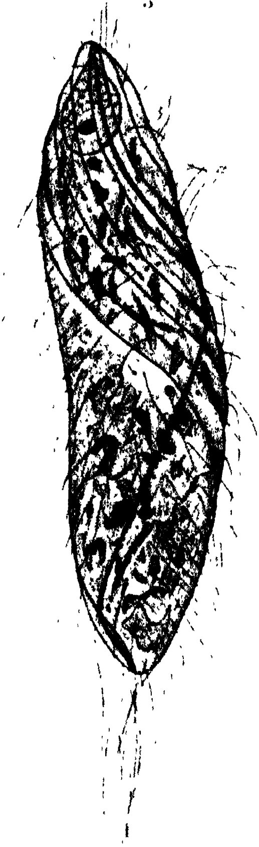

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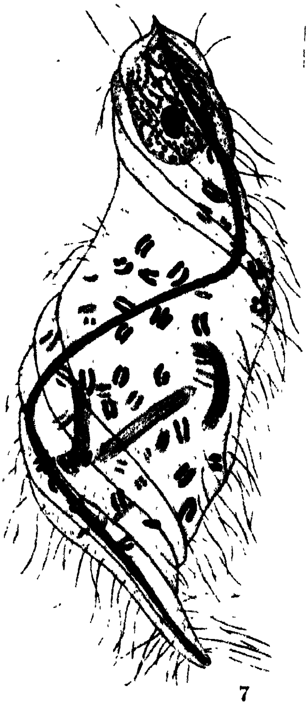

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PLATE 20

Fig. 8. An early prophase; chromatin organizing into strands; axostyle still remains attached to the blepharoplast.

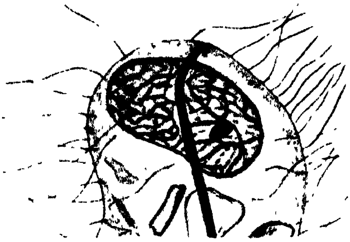
Fig. 9. Prophase. Chromosomes formed. Centroblepharoplast dividing, paradesmose between two. Four flagellar cords are attached to each. The small diagram in the upper left shows the condition of the centroblepharoplast, which lies under the nucleus.

Fig. 10. Prophase. Chromosomes organized; axostyle loose in endoplasm.

Fig. 11. Spindle. Small axostyles growing. The paradesmose appears over the nuclear membrane.

Figs. 12–14. Spindles, showing spindle fibers, growing axostyles, centroblepharoplasts at poles, and flagellar cords.

Fig. 15. Anaphase.



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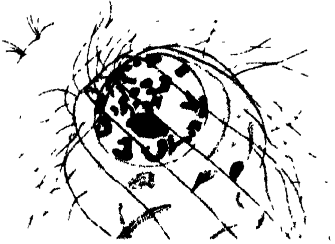

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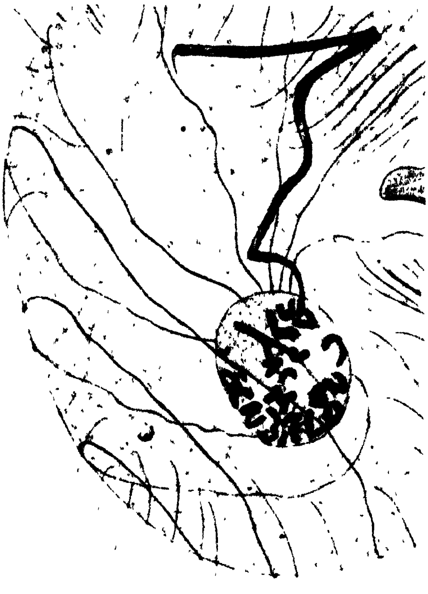

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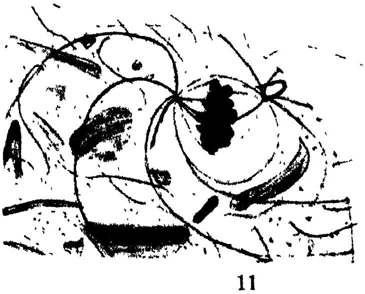

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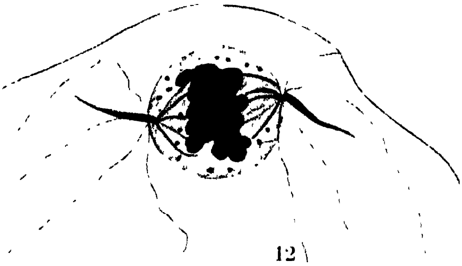

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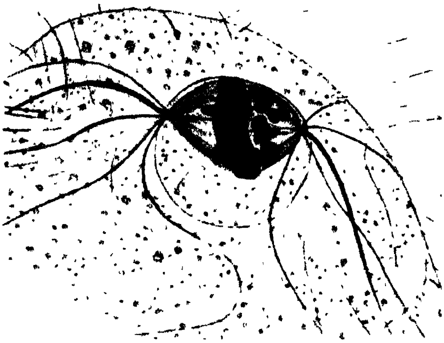

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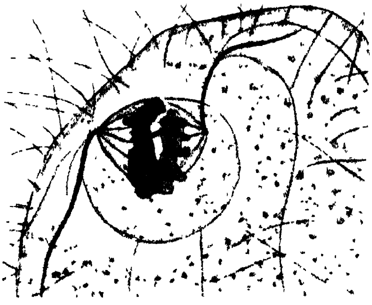

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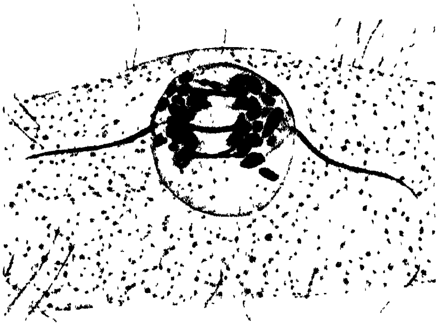

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PLATE 21

Fig. 16. Anaphase. The centroblepharoplast has given off the centrosome and the blepharoplast divided into two; to one of these (the primary) one cord and the axostyle are attached, to the other (secondary) three cords.

Fig. 17. Telophase. Blepharoplasts divided.

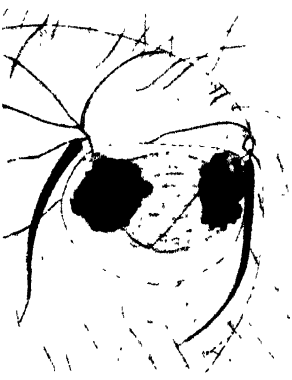
Fig. 18. Later telophase. Chromosomes loosening from knot. Wood particles still in endoplasm. An enlargement of the left nucleus is given in plate 22, figure 47.

Fig. 19. Late telophase. Chromosomes still further loosened from knot. Wood particles still present.

Fig. 20. After division of body. Centrosome on nuclear membrane and rhizoplast connecting it to blepharoplast.

Fig. 21. After division of body. There are two blepharoplasts, and a rhizoplast connecting to the centrosome on the nuclear membrane.

Fig. 22. After division of body. The chromosomes are connecting and breaking up. The axostyle is long, and there are four flagellar cords. No wood in body, but adherent bacteria are abundant.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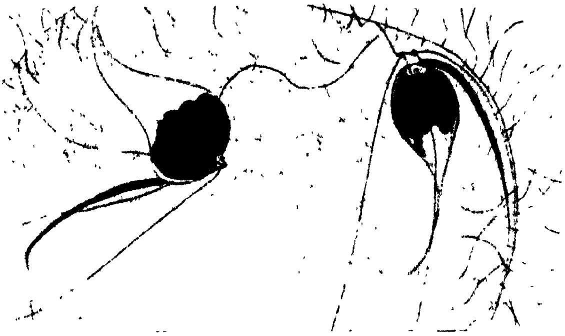
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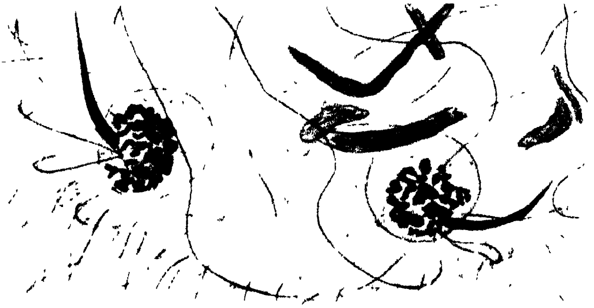
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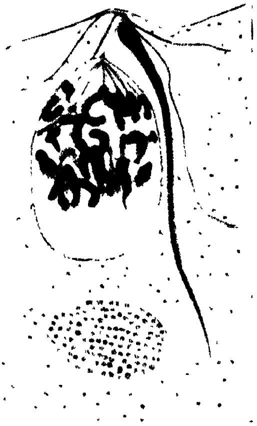
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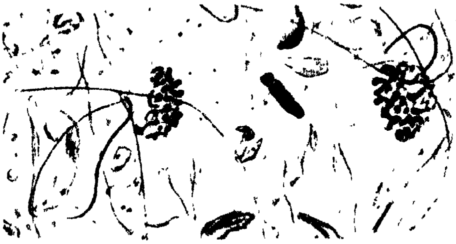
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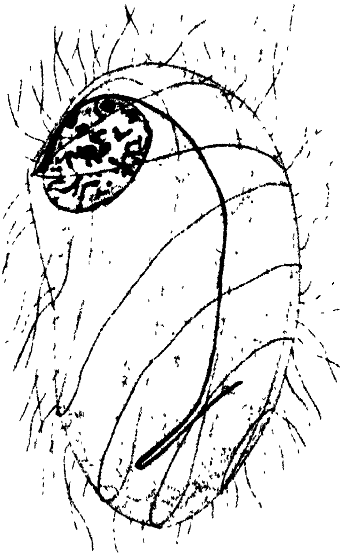
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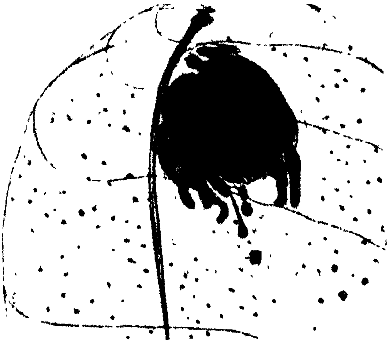
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PLATE 22

Isolated nuclei and chromosomes.

Fig. 23. Resting nucleus; vacuolated nucleolus in clear vesicle. Few chromatin granules on indistinct linin reticulum. Optical section.

Fig. 24. Chromatin granules collecting; vacuolated nucleolus.

Figs. 25–28. Figures showing condensation of chromatin and formation of threads.

Fig. 29. Threads forming. Some parts are deeply stained and solid threads; these are connected together by achromatic fibers.

Fig. 30. Similar stage to above, showing disconnected chromatin threads.

Figs. 31–32. Similar. Axostyle still in place.

Figs. 33–34. Condensation and thickening of threads. The nucleolus is disappearing. Some of the chromosomes are connected by linin threads.

Fig. 35. Splitting of chromatin threads. Axostyle gone.

Figs. 36–37. Contraction stages, suggesting synizesis.

Fig. 38. A looser condition of the chromosomes, seemingly emerging from a contraction phase such as foregoing.

Figs. 39–39 *b*. A later prophase, containing about 25 chromosomes. 39 *a* is an enlargement of figure 39. 39 *b* shows some chromosomes from figure 39.

Fig. 40. Chromosomes from the nucleus of figure 10.

Fig. 41. An early spindle. Flagellar cords divided four and four. The new axostyles are beginning to grow out.

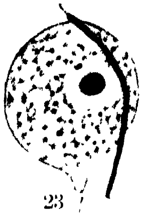
Figs. 42–44. Later spindles. Axostyles increasing in size, connected by fiber to blepharoplast. Figure 44 shows division into centrosome and blepharoplast.

Fig. 45. Anaphase.

Fig. 46. Some chromosomes of the nucleus of figure 22, plate 22.

Fig. 47. Enlargement of the nucleus of figure 18, plate 22.

Fig. 48. A nucleus of the telophase after plasmotomy.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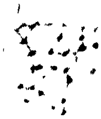
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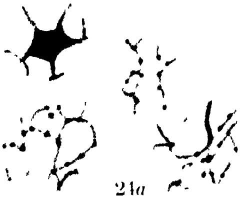
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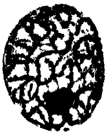
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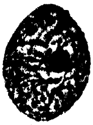
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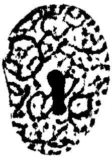
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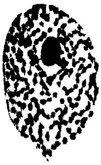
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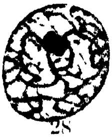
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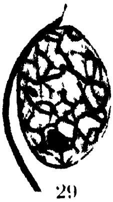
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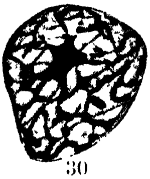
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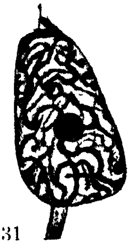
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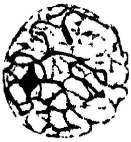
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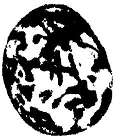
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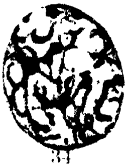
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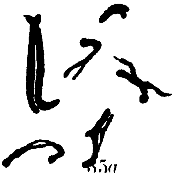
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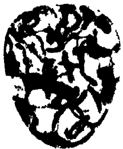
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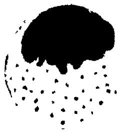
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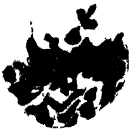
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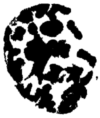
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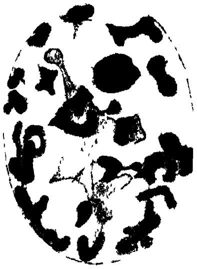
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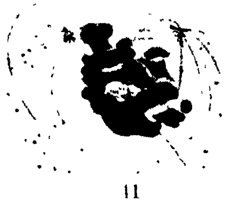
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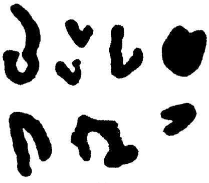
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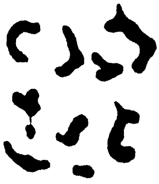
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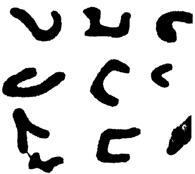
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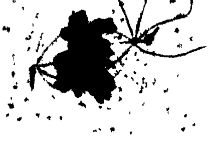
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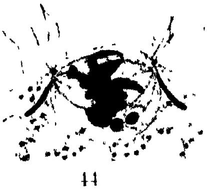
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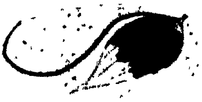
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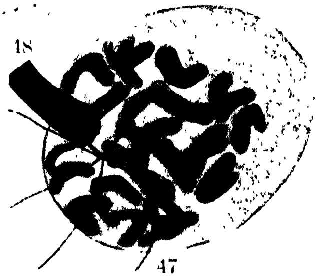
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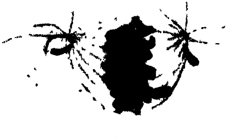
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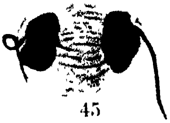
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KARYAMOEBA FALCATA, A NEW AMOEBA FROM THE HUMAN INTESTINAL TRACT

BY

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INTRODUCTION

The extent to which the intestinal tract of any mammal is parasitized by different species of Protozoa cannot be determined by casual or intermittent examinations. Continuous examination of successive stools is needed to bring to light the rarer infections and those which are less abundant in individuals. Continuity of examination also increases the chances of finding these at their recurrent but irregular maxima.

We know little of the extent to which a given mammalian host, such as man, may acquire the parasitic amoebas of other mammals, and still less of the conditions under which these cross-infections may be established and the factors which limit their persistence. The recent work of Kessel (1923) on cross-infections of intestinal amoebas from man to culture rats and mice has demonstrated the practicability of such transfers.

The literature of human amoebic infections, recently summarized by Dobell (1919), contains reference to "coprophile" amoebas, the inference being that such amoebas are primarily dwellers in faeces and only occasionally contaminate the food and invade the bowel of man. More critical investigation is needed to establish such an inference and demonstrate the specific identity of the amoebas concerned in the two habitats; on the one hand, in the human bowel with its stabilized conditions of temperature and pH, and, on the other, in the decaying faeces after discharge, with their markedly variable conditions of temperature and of decay with the resulting physical and chemical changes. There is no critical evidence as yet that the normal amoebas of soil and water ever exist in the motile phase in the human bowel. Some of them have been recovered by us in cultures from stools. They probably are able to pass through the bowel in cysts, but the identification of such cysts in faeces is as yet in an uncritical stage owing to lack of knowledge of the encysted phases of the amoebas of fresh water.

The purpose of this paper is to place on record an amoeba of human faeces in the human bowel of which no cysts have as yet been found. In this respect it resembles *Dientamoeba fragilis* described by Jepps and Dobell (1918), for which fully developed cysts with glycogen, chromatoidal bodies, and nuclear multiplication have not yet been discovered. In an earlier publication (Kofoid, 1923) we have figured an early phase of encystment in this species but its later phases have not, to date, been reported by any investigator of parasitic amoebas of man.

This absence of typical encysted phases in both of these amoebas is at least suggestive that their dependence upon the host or upon an ability to live in the bowel is at least of a different nature from that of the amoebas with typical cysts, such as *Endamoeba coli.* How they are transferred to new hosts, and their fate in the stool after discharge, are still unknown.

OCCURRENCE

The occurrences of this new amoeba in considerable numbers in fresh warm stools from three different persons, and from one of these persons in September and again in March, seem to make it certain that this is not a mere accidental contamination of man, but a normal inhabitant of the human bowel. If so, it is to be expected to appear occasionally, as has *Dientamoeba,* in surveys of human intestinal protozoan infections.

Three cases of infection of the human bowel by this amoeba have come under our observation. The first was in a lad (H) of thirteen, who also had infections of *Endamoeba dysenteriae, E. coli, Endolimax nana, Councilmania lafleuri, Dientamoeba fragilis,* and *Blastocystis hominis.* This case was examined by us ten times between September 17 and October 17, 1923, the available material including two warm stools in thermos bottles. *Karyamoeba falcata* sp. nov. was present in four of these stools including and following two warm stools after a saline purge. A reëxamination of seven stools, from March 11 to 17, 1924, revealed *K. falcata* in small numbers only in one warm stool following a saline purge. The case had received the emetin bismuth iodide-neo-arsphenamine treatment in the previous year. All the infections found on the first examination, except *E. dysenteriae,* were found in these reëxaminations along with *Karyamoeba.*

The second case was the lad's mother (Mrs. H), aged 45, who was examined by us seven times between September 11 and October 17, 1923. *Karyamoeba* was found only on the last examination, concurrently with *E. dysenteriae,* in a warm stool in a thermos bottle following a saline purge. Stools from this case were reëxamined by us in March, 1924 and were negative for *Karyamoeba.*

This second case was born in Ohio, and had lived in California for twenty years. The son was born in California and had lived in San Francisco and Berkeley, except for nine months near Escondido in San Diego County in Southern California. Neither parent had lived or traveled in the tropics but the father had been in Alaska. The father and a six year old sister did not show this infection on seven and eight examinations respectively, though the sister had *E. dysenteriae,* and a history of enlarged joints from the age of ten months.

The third instance was detected in a woman (Mrs. T) aged 50, who was examined by us seven times between October 10 and 26, 1923. *Karyamoeba* was detected in two of the stools, including one warm stool in a thermos bottle. This case carried infections of *E. dysenteriae, E. coli, C. lafleuri,* and *Blastocystis hominis.* The patient had arthritis. She reported that she had always resided in this locality. In March she received treatment for amoebiasis, and, upon reëxamination, *Karyamoeba* was not once detected in the stools inspected.

PSEUDOPODIA AND LOCOMOTION

Motile amoebas in fresh smears from a warm stool in a thermos bottle following a saline purge were observed on the electric warm stage for several hours. The stool was from the H case, and on the day of examination contained cystic and precystic but no motile phases of *Councilmania lafleuri,* motile *Endamoeba dysenteriae* of a small race, and *E. coli* with characteristic granular pseudopodia and sluggish movements.

In addition to these there were other amoebas some of which had visible nuclei of the type found in *Karyamoeba.* Some of these were small, 10–12 microns, and others were of a larger size up to 30 microns in diameter. Since they did not conform in appearance or behavior to the other known species in the stool, and since some of them in life had nuclei of the *Karyamoeba* type, and since characteristic *Karyamoeba* were found in stained slides from the stool, we concluded that we were observing the motile forms of this species.

There was a great abundance of motile forms of this species, sometimes as many as twelve in one field in the smears. These were continuously, or intermittently, active in many cases, so that it was possible to study the activities of the motile phase in a number of individuals, and thus to compare them with the activities of *Endamoeba coli* and *E. dysenteriae* on the same slide.

The general form of the motile phase of *Karyamoeba falcata* is elongated, about twice as long as wide, with frequent changes in outline, especially when in contact with obstructions. These changes result from the abrupt origin of pseudopodia at other points than the then anterior end and involve frequent and rapid shifts in the axis of progression and the form of the body (fig. A). In sharp contrast with this, *E. coli* tends to move for a longer time in one direction and to taper more posteriorly, and often exhibits an adhesive cone of attachment at its posterior end.

In *Karyamoeba* the pseudopodia (fig. A) are at first rather narrow, from 1.0 to 1.5 the diameter of the nucleus in width, increasing slightly during formation to 2–3 nuclear diameters, especially in the larger amoebas. With the inflow of endoplasm the pseudopodium may ultimately become the main part of the body and be still further expanded. They begin to fill with endoplasm shortly after their length is extended to a distance equal to their basal diameter. At first the pseudopodia are clear, not granular, as in the body proper. They have not, however, the strikingly hyaline or crystal-clear appearance of the pseudopodia of *Councilmania* or that of the clear

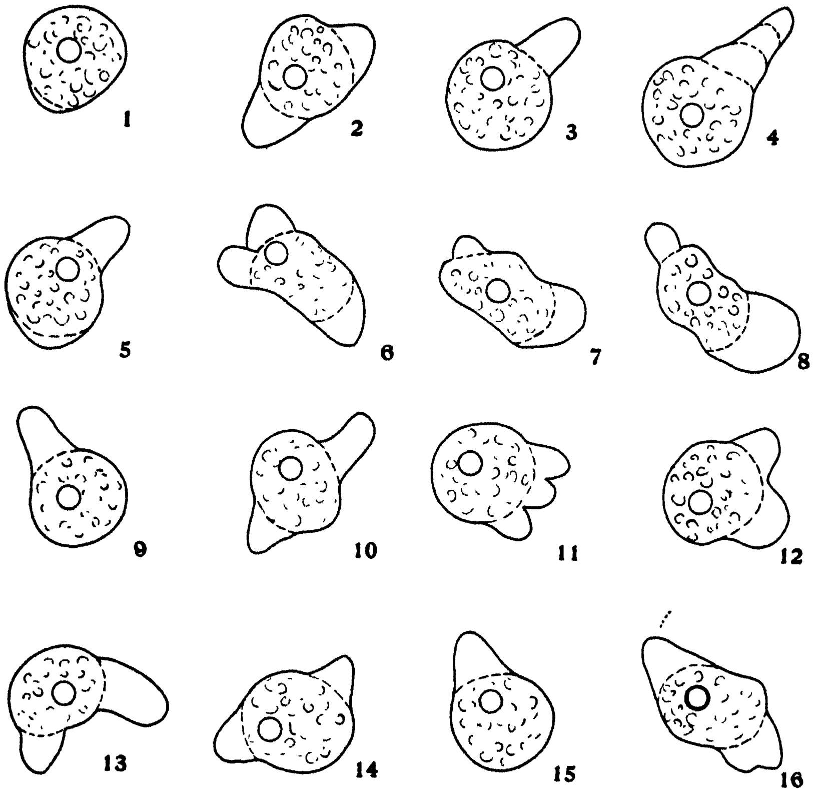

Fig. A. 1–16. Outline sketches of *Karyamoeba falcata*, in successive phases of locomotion, drawn from faecal smear on the electric warm stage during a period of fifteen minutes. The orientation with reference to the observer is the same in all of the figures. Note the reversal in direction between 1 and 2, 4 and 5, and 15 and 16, and the wide range of margins on which pseudopodia form. Note also the persistence of the first pseudopodium during the formation of the second or even the third (11). Vacuole forming around the nucleus in 16.

pseudopodia of *Endamoeba dysenteriae*. They are more opaque, darker, and are more quickly invaded by the still darker and more definitely granular endoplasm than is the case with the two amoebas named. They are unlike the pseudopodia of *E. coli* in that they are clear, rather narrower and smaller, more quickly and more frequently formed, and are not filled with endoplasm. Another marked difference from *E. coli* is the absence of any posterior, conical, adhesive area, such as may appear in that species during locomotor activity. They are also less elongated.

Karyamoeba falcata usually forms but a single pseudopodium at one time but, owing to the rapidity of formation of successive pseudopodia at adjacent or at more or less widely separated points on the body, there may at times be as many as three pseudopodia present. Of these usually one is forming and one or both of the others retracting (fig. B 2).

There is one rather striking feature of pseudopodial formation often seen in the more sluggish individuals. In these the area of peripheral solation is often of less diameter than either the posterior body or the anterior pseudopodium, with the result that there is a temporary constriction nearly midway of the animal, as though the organism were about to divide. This constriction quickly disappears as the posterior residuum is readjusted to the advance of endoplasm into the pseudopodial area.

The activity of this species is very pronounced, and its agility in creeping through narrow passages in the faecal débris and in vigorously pushing its way through masses of bacteria is very noticeable. It remains active for several hours in vaselined slides on the electric warm stage, but rounds up and becomes quiescent sometimes before the much more sluggish *Endamoeba coli* ceases locomotive activity, though it persists in activity longer than does *E. dysenteriae*.

MORPHOLOGY

No encysted individuals of this species have been found, consequently our discussion is restricted to the morphology of the motile phase only, and is based upon amoebas in smears prepared by the Schaudinn-iron-haematoxylin method.

This species appears to be more sensitive to stimuli than are other amoebas of the human stool since by far the greater number of individuals in our preparations are rounded up, even when fixed immediately from warm stools. Only rarely can we find (pl. 24, fig. 16) an extended pseudopodium in stained preparations.

THE ECTOPLASM

The cytoplasm in many individuals is sharply divided into ectoplasm and endoplasm (pl. 23, figs. 2, 5; pl. 24, figs. 12–14). In a rounded-up individual showing a large amount of distinct ectoplasm, the computed volume of the ectoplasm is thirty per cent of the total volume of the amoeba. In such rounded-up amoebas the ectoplasm is very sharply differentiated from the enclosed endoplasm, as it is also in one (pl. 24, fig. 16) with protruded pseudopodium. In this pseudopodium a more deeply siderophile internal portion with some granulations is revealed by staining. A very large individual (pl. 24, fig. 14) with a rather wide zone of ectoplasm also exhibits ectoplasmic granulations which locally merge into the endoplasm. In life the ectoplasm is distinctly of the hyaline type though quickly invaded by endoplasm. The ectoplasm often appears to envelop the entire body.

The periphery of the ectoplasm is covered by a very definite pellicle (*pell.*, fig. B) staining differentially a darker tone than the underlying ectoplasm. It is about as thick or even thicker than that on *Endamoeba gingivalis* (see Kofoid and Swezy, 1924). Amoebas of this species are found (pl. 23, fig. 7) in which no traces of any ectoplasm other than the pellicle can be detected. In these the endoplasm in its normal structure continues to the pellicle or to the hyaline subpellicular layer. The periphery shows clearly the narrow, clear zone first noted by Bütschli (1894) and later more clearly defined by Gruber (1912), not confirmed by Jennings (1904), but reaffirmed by Schaeffer (1917, 1920). Beneath the narrow but definitely differentiated pellicle we find in stained amoebas of this species a sharply delimited clear layer of uniform thickness which intervenes between the pellicle and the more granular ectoplasm. The uniformity and constancy of this structure is striking. We do not find it in *Endamoeba dysenteriae*.

ENDOPLASM

The endoplasm of this species is rather coarsely granular both in life and in stained preparations. Most individuals are crowded with vacuoles strictly confined to the endoplasm. In these amoebas the endoplasm may be fairly homogeneous in stainability with fine alveolations (pl. 24, fig. 14), or retain the stain about the nucleus more than in the periphery (pl. 23, fig. 1). In individuals having few vacuoles (pl. 24, fig. 17) the endoplasm may appear to be condensed. These conditions are suggestive of a high metabolic rate and of the agency of the vacuoles in the metabolic processes reflected in varying stainabilities and alveolations of the endoplasm. Coupled with the considerable numbers present in the stools, there appear to be grounds for regarding this organism as adapted to thrive in human faeces in the bowel.

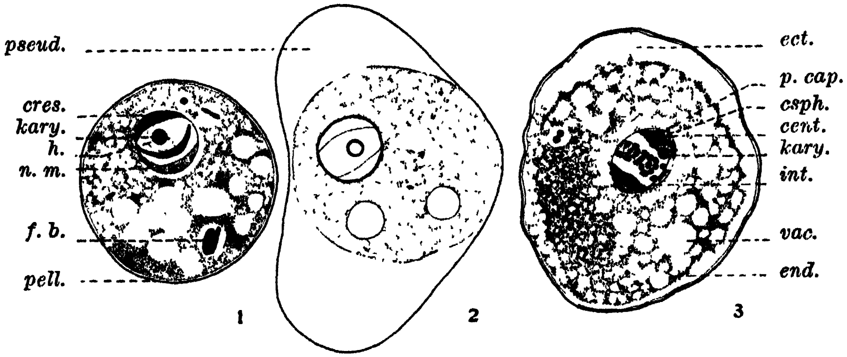

Fig. B. *Karyamoeba falcata* gen. nov., sp. nov. × 1500. Abbreviations: *cent.*, centriole; *cres.*, crescentic peripheral siderophile body; *csph.*, centrosphere or halo about centriole; *ect.*, ectoplasm; *end.*, endoplasm; *f. b.*, food body; *h.*, karyosomal halo; *int.*, intradesmose; *kary.*, karyosome; *n. m.*, nuclear membrane; *p. cap.*, polar cap; *pell.*, pellicle; *pseud.*, pseudopodium; *vac.*, vacuole. 1. Rounded-up amoeba with pellicle, hyaline zone, but no other ectoplasm. Endoplasm vacuolated, a food body in one vacuole only. Nucleus enveloping halo, excentric spherical karyosome with clear halo, three crescentic siderophile peripheral masses. 2. Amoeba in locomotion with two large hyaline pseudopodia. From life. 3. Amphiaster phase in mitosis with meridional intradesmose with terminal centrioles in centrosphere or halo at apex of polar cap. Persistent karyosome. Chromosomes in end-to-end relation in equatorial plate.

Fluid-filled vacuoles (*vac.*, fig. B) characteristically crowd the endoplasm. These are usually without food bodies. The enveloping cytoplasm is rather coarsely and uniformly granular and minutely alveolar. The vacuoles are spherical to ellipsoidal, two to three, rarely five or six microns in diameter, and present a marked but not complete uniformity in size and distribution throughout the cytoplasm. In a few instances only (pl. 24, figs. 14–16) are definite food bodies found in these vacuoles. In one instance a short rod-like bacillus (fig. 13) was found in an elongated vacuole, and in others spheroidal structures of unknown nature. These vacuoles do not differ in appearance or structure from those without such bodies. It would appear from these findings that this amoeba may feed, by osmosis, on the fluid contents of the bowel. It is hardly possible that the fluid could be engulfed without at the same time including the omnipresent bacteria.

In addition to the vacuoles, there are present in the endoplasm of many individuals a few scattered siderophile granules, rodlets, or threads (pl. 23, fig. 3; pl. 24, fig. 16) which are not enclosed in food vacuoles, but appear to lie free in the cytoplasm, and therefore to present products of metabolism rather than food prior to that process. Some of these are spherules (figs. 12, 16), others are elongated (fig. 13), while others are even filamentous (fig. 3). Some stain with greater distinctness than others and it is possible that they are all phases of the transformation of one kind of material. In a few amoebas of this species (pl. 23, fig. 6) the endoplasm is deeply stained and is itself crowded with many dark metaplasmic structures, some of which are elongated with rounded ends, others slender and almost filar, while still others are rod-like. These structures are not enclosed in food vacuoles and do not seem to be food bodies. They appear to represent an extreme phase of the formation of metaplasmic material not unlike the chromatoidals of cysts prior to and during the first mitosis. It is to be noted that two such amoebas (figs. 13, 14) are in the metaphase, but not all metaphases have these bodies.

One puzzling and possibly suggestive phase of this phenomenon is the juxtaposition of some of these bodies to the nucleus and their approximation in form to the crescentic siderophile bodies inside of the nuclear membrane (pl. 23, figs. 3, 10).

NUCLEUS

This is strikingly different from those of all other amoebas of the human stool, and this difference constitutes the basis for the generic distinction of this amoeba. The nucleus is extraordinarily variable in size, ranging from four (pl. 23, fig. 5) to eight (pl. 23, fig. 10) microns in diameter and with a mode at five or six. The volume of the nucleus is not proportional to that of the cytoplasm, the largest nucleus found occurring in one of the smallest amoebas (fig. 10). These size relations indicate a correlation of the size of the nucleus with considerable changes in metabolic activity, rather than with the volume of the cytosome. The small amoeba with the large nucleus (fig. 10) has almost no vacuoles. Large amoebas with small nuclei may have many vacuoles.

The *nucleus* is uniformly spherical except in the later stages of mitosis and has not been found in deformed or compressed condition as has the nucleus of *Endamoeba gingivalis* (see Kofoid and Swezy, 1924). A cloud of more deeply staining, vacuole-free endoplasm sometimes surrounds it (pl. 23, fig. 1). In a few instances, one or two crescentic, siderophile bodies, either with or without an enveloping vacuole, are applied to, or lie near its outer surface (pl. 23, figs. 3, 10). In a number of instances the nucleus lies in a completely or a partially enveloping clear area (pl. 23, fig. 2) or vacuole. There is no structural modification to indicate that this is an artifact due to shrinkage. It is possibly normal, but has not been observed in life.

The nucleus consists of a homogeneous, lightly staining nuclear membrane, peripheral chromatin in the form of stout, or slender, crescentic, siderophile structures, and other small amounts irregularly applied to the membrane included in the general term peripheral chromatin, and a neatly spherical, subcentral karyosome with or without a clear halo.

The *nuclear membrane* (*n. m.*, fig. B 1) although not encrusted uniformly with chromatin and often enclosed in a fluid-filled vacuole is not deformed or collapsed. It is not deeply stained and is homogeneous in structure. This with the absence of distributed peripheral chromatin and the limited amount of interzonal siderophile granulations gives a remarkably clear aspect to the nucleus as a whole and sets off the siderophile crescents with exceptional distinctness.

The *peripheral chromatin* of this species is very characteristic in form and appearance. It is grouped in two, or one to three, large, crescentic masses (*cres.*, fig. B 1), tapering at the two poles to more or less slender tips, and extending for half a circumference more or less on the nuclear membrane. In a few cases these bodies are stout with blunt ends (pl. 23, fig. 10) and in others they are composed of two to five segments in a row (pl. 23, fig. 1). They appear, as a rule, to be applied closely to the nuclear membrane, but to this some exceptions occur. In a few instances (pl. 23, figs. 4, 7) a slender, siderophile thread connects opposite ends of the two bodies. We are unable to establish a relation of this peripheral chromatin to the intradesmose though such a relation is suggested. There is in addition, in some instances, a small amount of scattered chromatin.

The substance composing these bodies is siderophile and homogeneous. In several instances, including a number not figured by us, we find (pl. 23, fig. 10) crescentic bodies of similar size, shape, structure, and stainability, lying in the cytoplasm detached from the nucleus, and other elongated threads of smaller sizes and more curvature scattered in the cytoplasm (pl. 23, fig. 3). In still other amoebas a siderophile body of stout form not unlike those inside of the nucleus is applied closely to the outside of the nuclear membrane. In some of the more flattened siderophile structures the substance has a lighter tone, as a result of the thinner mass of material. It is evident that there is much more siderophile material in the nucleus than that which forms the chromosomes.

The significance of these concurrent phenomena is not clear but they suggest the passage of siderophile substance from the inside of the nucleus out into the cytoplasm. Taken in conjunction with the circumnuclear vacuole, they indicate a high state of metabolic activity involving intranuclear material.

The *intermediate zone* between the karyosome and the peripheral chromatin of the nucleus of this species is either entirely clear, or faintly, or coarsely clouded with stainable material. We have not found it to be normally zoned in this species in any significant manner as it is in *Endamoeba dysenteriae* or in *E. gingivalis.*

The *karyosome* (*kary.*, fig. B) is strikingly characteristic in this species. It is excentric in location in the nucleus, nearly spherical in form, 1.5 (1.2–2.0) microns in diameter, homogeneous in structure, deeply staining, and is generally centrally located in a clear halo (*h.*, fig. B) whose thickness is from 0.5 to nearly 1.0 diameter of the karyosome. In a few instances we were not able to establish the limits of the halo (pl. 23, fig. 10).

We were not able to find the karyosome in one of two nuclei of a binucleate amoeba (pl. 24, fig. 17) although there was a diffuse, deeply staining region in it, possibly a newly forming karyosomal sphere. In several other instances (pl. 23, fig. 5) no karyosome could be found in the nucleus. In all amoebas in phases of mitosis (pl. 24, figs. 12–16) in which decolorization was adequate, the karyosome could be found on one side of the equatorial plate although somewhat smaller than in resting nuclei. These findings lead us to infer that the karyosome is neither divided nor entirely torn down at mitosis, but persists, at least in large part, and is passed to one of the daughter nuclei, while the other daughter nucleus organizes a new karyosome.

A *centriole* or *centrosome* was found in two instances only in resting nuclei (*cent.*, fig. B; pl. 23, fig. 1) as a small, spherical, deeply stained granule applied to the side of the karyosome (fig. B 1). It is obvious that such a structure would be visible only in full lateral view, and, owing to its minute size, only in a small proportion of the possible views of the karyosome. We interpret it tentatively as the centriole and have no evidence as to the permanence of its location in the resting nucleus. At the anaphase, both of the centrioles and the single persistent karyosome are rather far apart (pl. 24, fig. 14). We have no evidence that the centriole is included within, or emerges from the karyosome, at any stage in the mitotic process.

REPRODUCTION

In the entire absence from the stools of developed cysts of this species the only evidence we have of any method of multiplication is the occasional detection of an amoeba whose nucleus is in some phase of mitosis, and the finding of binucleate amoebas (pl. 24, figs. 12–17). These are adequate evidences of multiplication by binary fission in the bowel. Our mitotic material came wholly from one fresh stool fixed while still warm. In a few cases only have we found amoebas which were possibly in a precystic stage. One such amoeba (pl. 23, fig. 8) was perfectly spherical, had a heavier pellicle than usual, and had few vacuoles.

MITOSIS

Our findings are adequate for a description of the main features of the process of mitosis but details are lacking, especially in the early prophases and the telophase. We have a sufficient number of stages to enable us to define definitely the type of mitosis and to approximate the number of chromosomes. This species has a slender meridional intradesmose, connecting, minute centrioles, very large polar caps, and a large number of chromosomes, and is thus clearly of the *Vahlkampfia* type as defined by Jollos (1917).

It is probable that the earliest prophases are represented by amoebas which have a nucleus with a very large and slightly decolorized karyosome (pl. 23, fig. 1) in which certain peripheral chromatin is breaking up into fragments and the centriole appears near the karyosome. We have found a large number of amoebas in later stages of mitosis in which (pl. 24, figs. 12–17) there is a slender intradesmose in a meridional location joining the two minute, deeply staining centrioles at the pole of the lenticular polar masses. These masses are large and may be asymmetrically shaped with reference to the intradesmose, flattened or concave on the proximal surface, but convex and closely fitted against the nuclear membrane. A smaller somewhat decolorized karyosome persists in these amoebas after the formation of the polar caps. It appears from this that these caps are not formed at the expense of the karyosome but probably from the large crescents. The origin of the centriole is uncertain. We find it near the karyosome in the prophase. The karyosome, however, reduced in size and stainability, persists, and the siderophile crescents vanish into the polar caps.

At the *metaphase* (fig. B 3; pl. 24, figs. 15, 16) the massive, slightly asymmetrical, siderophile polar caps lie at opposite poles. Midway between them lies a band of deeply staining material (pl. 23, fig. 11) which on being heavily destained is resolved into the now reduced spherical karyosome and a number of chromosomes. In most instances the karyosome appears to be crowded against the nuclear membrane, and to have no visible connections with any mitotic structures. Both it and its halo are slightly reduced in size, and it decolorizes more than the larger polar caps.

At the poles we can detect in some instances (fig. B 3; pl. 24, figs. 13, 15) a lighter halo or zone surrounding the centrioles and indenting the polar caps as lighter areas, or possibly 'centrospheres.' The name centrosome might fittingly be applied to the combined centriole and its encircling 'centrosphere.'

The *intradesmose* (*int.*, fig. B 3) is a uniformly slender, siderophile fiber which follows a meridional course on the inner face of the nuclear membrane and connects the two centrioles at the poles. Its course is usually direct, but in a few instances (pl. 24, fig. 14) it has a sigmoid curvature. Its distal ends pass over the surface of the deeply stained polar caps, and it is followed across them with difficulty. It is recognizable by its seemingly deeper stainability and elevation above the surface at the cap. We have no evidence as to the method of origin of the intradesmose except one early prophase (pl. 24, fig. 12). By analogy from other amoebas and from this stage one would expect that this structure would be spun out between the centrioles as they move to the poles.

We have found no late anaphases or telophases. The latest stage available is an early anaphase (pl. 24, fig. 16) marked by the scattering of the chromosomes from the narrower equatorial band of the earlier phases.

A late prophase (pl. 24, fig. 12) shows a meridionally located but short intradesmose joining the still rather tapering tips of the crescentic polar caps. The slightly enlarged ends of this intradesmose are still at the tips of the crescents and cannot be traced to the mid-polar area. The intermediate area between the polar caps is occupied by a very vaguely staining mass of material in which one can trace the faint and rather ragged outlines of elongated structures which may be the shortening daughter chromosomes. It is impossible to count them at this stage.

The *chromosomes* appear most clearly during the metaphase, in which they are assembled in an increasingly orderly array in a narrow double equatorial plate as the amphiaster phase culminates. They appear to have been split prior to this assembling, and to emerge in an end-to-end or pseudo-telosynaptic juxtaposition, which may be interpreted as the final separation at one end, of two daughter chromosomes parted by the typical process of longitudinal splitting. At this stage they are ellipsoidal in form, and range from sub-spherical to elongated, ellipsoidal shapes. There are also differences in degree of separation.

The number of chromosomes is approximately twenty. Generally about ten can be clearly made out in the nearer face, but the deeper-lying ones are not easily counted. In the later metaphase (pl. 24, fig. 16) the chromosomes and chromosome pairs are scattered rather widely between the polar caps. The conditions at the telophase are unknown.

In the reorganizing daughter nuclei (pl. 24, fig. 17) the karyosome persists in one nucleus, and is not as yet clearly reorganized in the other. Each nucleus has one large peripheral crescent (polar cap) and several smaller masses lie elsewhere on the membrane. The chromosomes are probably dispersed in the vague granular masses in the periphery of the nucleus. No trace can be found of the centriole or intradesmose in either nucleus.

RELATION TO THE HOST

Since all three of the patients in whom this amoeba was found had concurrent infections of *E. dysenteriae*, it is not possible to isolate or determine the consequences of this infection upon the human host.

GENERIC CHARACTERS OF KARYAMOEBA GEN. NOV.

This genus exhibits in the motile phase the following complex of characters: clear pseudopodia; nucleus with neatly spherical karyosome excentric in location, peripheral chromatin clumped in a few large, elongated masses, nuclear membrane distinct, chromosomes numerous, parasitic in the intestine of mammals.

Type species **Karyamoeba falcata** sp. nov.

SPECIES CHARACTERS

Pseudopodia less clear than in *Endamoeba dysenteriae* and *Councilmania lafleuri,* rather promptly invaded by endoplasm but not at once in the forming pseudopodium as in *E. coli,* blunt, one to two nuclear diameters in width at base, rarely longer than wide; pellicle very thick; peripheral chromatin massed in one or two, rarely more, crescentic clumps, semicircular in extent, on the nuclear membrane; halo around the karyosome normally present; no trace of differentiated concentric zones in nucleoplasm; chromosomes about twenty in number. In faeces in the human intestine in the motile phase. Cysts not as yet discovered. Types in collection of University of California, slide nos. 27680, 27708, 27737, 28087.

RELATIONSHIPS

The review of the genus *Vahlkampfia* recently published by Nöller (1922) makes possible a comparison of our species with those previously published in that genus or assigned to it by him. The genus *Vahlkampfia,* as defined by Chatton and Lalung-Bonnaire (1912) and emended by Calkins (1913), is characterized by a single pseudopodium, a single nucleus with a large, centrally located, spherical karyosome, and a very small amount of evenly distributed peripheral chromatin. The nuclear structure of *Karyamoeba* differs from that of all other known parasitic amoebas.

Karyamoeba falcata is related to *Vahlkampfia* by reason of its nuclear structure at mitosis as defined by Jollos (1917), especially by the presence of a slender intradesmose with terminal centrioles, each in a halo. It differs from *Vahlkampfia* in the occurrence rather frequently of more than one pseudopodium, in the larger size of the polar caps, in the presence of the excentric spherical karyosome persistent throughout mitosis, and in the distribution of the large amount of peripheral chromatin in large masses.

Systematic Position

In view of the growing practice among protozoologists of utilizing the finer cytological characters of the nucleus for systematic distinctions and the importance of such characters in the Amoebina and especially in parasitic intestinal amoebas, we utilize these characters in this instance as the basis of generic distinction. There is no other amoeba in the literature known to us which has the nuclear structure of this organism, specifically the crescentic siderophile structures in which the peripheral chromatin is grouped.

There is no trace of a blepharoplast in the cytoplasm and no flagellate phase has been found in the stools of any one of the three cases we have studied. In the absence of a blepharoplast and a flagellate phase, it is judged to be one of the Amoebidae Doflein (1916), and is placed in a genus coördinate with the genera *Endamoeba* and *Vahlkampfia*. It differs from all other genera in this family in the possession of the crescentic, siderophile structures.

It appears to be more closely related to *Vahlkampfia* than to the other genera in the size and structure of the polar caps. It differs from *Vahlkampfia* in the small size and excentric location of the karyosome and in the abundance and crescentic massing of the peripheral chromatin. It is not possible to include it in that genus without making a heterogeneous assemblage of nuclear types therein. We therefore place it in a special genus, *Karyamoeba* gen. nov.

SUMMARY

Three instances of the infection of man by an amoeba *Karyamoeba falcata* gen. nov., sp. nov. in the faeces are here recorded. All three were accompanied by *Endamoeba dysenteriae* so that relations to the host were complicated. This amoeba persisted after treatment for amoebiasis with no clinical signs of pathogenicity. It was observed in one case in September and again in March. It does not appear to be a coprophile contamination but rather a persistent inhabitant of the human bowel. It occurs in the motile phase only. Mitosis and binucleate amoebas and rounded-up (precystic?) phases were found.

This amoeba is characterized by one, rarely two or more, blunt, hyaline pseudopodia. Ectoplasm and endoplasm are sharply separated. There is a heavy peripheral pellicle, a narrow, clear, ectoplasmic

zone beneath it, and an inner ectoplasmic mass of greater or lesser volume sharply set off from the more granular endoplasm. The endoplasm is coarsely vacuolated; food bodies are rare in these vacuoles.

The nucleus contains one or two, rarely more, large, crescentic, siderophile masses, applied to the nuclear membrane and little peripheral chromatin otherwise applied to the membrane. There is a neatly spherical, excentric karyosome in a halo adjacent to which a minute centriole is sometimes found. There is no concentric zoning of the intermediate region.

Mitosis proceeds by the emergence of polar caps, apparently from (?) the crescentic masses, the division of the centriole and the migration of the daughters to the poles over the outside of the polar cap on whose summit the centriole lies in the center of a lighter halo. The centrioles are joined by a slender, thread-like, meridional intradesmose. Chromosomes emerge probably from peripheral chromatin as elongated masses in the area between the polar caps. These shorten up into two rows in an end-to-end arrangement in the amphiaster stage. There are approximately twenty chromosomes. Mitosis is of the *Vahlkampfia* type.

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PLATE 23

All figures of *Karyamoeba falcata* gen. nov., sp. nov. × 2500.

Camera lucida drawings from faecal smears fixed in hot Schaudinn's fluid and stained in iron haematoxylin.

Fig. 1. Rounding-up amoeba with pellicle, thin hyaline layer but no ectoplasmic pseudopodia. Siderophile cloud in endoplasm near the nucleus. Halo around centriole at side of enlarged, less deeply staining karyosome. Siderophile crescents on nuclear membrane not massed. Possibly a very early prophase. Vacuoles without food bodies.

Fig. 2. Amoeba with two superposed pseudopodia. Nucleus in a vacuole. Subcentral karyosome in its halo. One large crescent. Food bodies in two vacuoles only.

Fig. 3. Small rounded-up amoeba with filar and crescentic siderophile structures in the endoplasm, not enclosed in vacuoles. Nucleus in a vacuole, excentric karyosome in halo and one elongated crescent.

Fig. 4. Elongated amoeba with retracting pseudopodium invaded by endoplasm. Nucleus in thin halo, large excentric karyosome in halo, two peripheral siderophile bodies with opposite poles connected by a siderophile thread. Traces of small food bodies in three vacuoles.

Fig. 5. Rounded-up amoeba with sharp demarcation between ectoplasm and endoplasm. Small nucleus (recently divided?) with no karyosome and scattered, peripheral, subcrescentic siderophile bodies. Several small siderophile granules in the endoplasm but not in vacuoles.

Fig. 6. Rounded-up amoeba with sharply delimited endoplasm and wide peripheral ectoplasmic zone. Endoplasm crowded with flakes, rods, granules, and subcrescentic siderophile bodies. Nucleus approaching amphiaster stage with unequal polar caps, deeply stained equatorial mass in which darker bodies (chromosomes) are indicated. Meridional intradesmose along lower edge of the nucleus. Persistent karyosome in margin of equatorial mass. No vacuoles.

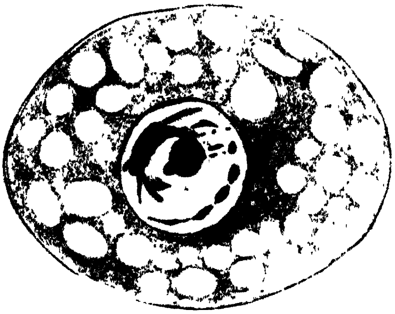
Fig. 7. Rounded-up amoeba with no ectoplasm except pellicle and subpellicular hyaline layer. Nucleus with three crescentic, siderophile, peripheral masses, two of which are connected by a dark thread. Excentric spherical karyosome in a large halo. Food body in one vacuole only. Siderophile sphere in endoplasm.

Fig. 8. Rounded-up amoeba with few vacuoles. Nucleus in vacuole with two crescents and karyosome with halo. No ectoplasm and no clear layer. Possibly precystic.

Fig. 9. Rounded-up amoeba with two pseudopodia almost withdrawn. Ectoplasmic zone wide, deeply stained. Endoplasm full of vacuoles, one only with a food body. Nucleus with two crescents and some other peripheral chromatin masses. Excentric karyosome with halo.

Fig. 10. Very small amoeba with very large nucleus. Nucleus with central karyosome, clear nuclear area, three crescentic masses. Applied to the exterior of the nucleus is a vacuole containing a stout crescentic siderophile mass. No ectoplasm. Only one vacuole in the endoplasm.

Fig. 11. Rounded-up amoeba with deeply staining ectoplasm, highly vacuolated endoplasm with seven large elongated flakes of siderophile substance, one on end against the nucleus. Nucleus in late prophase with polar caps and equatorial mass with persistent karyosome.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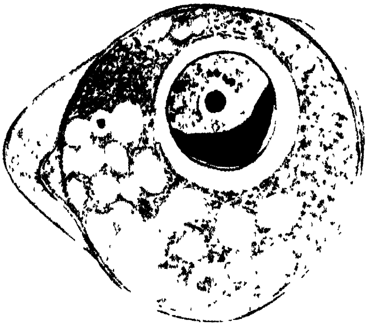
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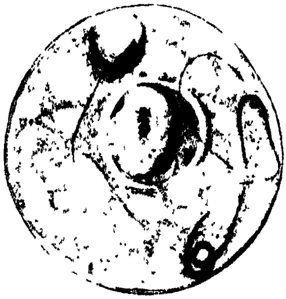
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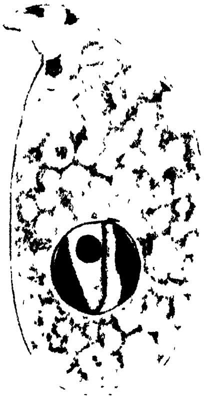
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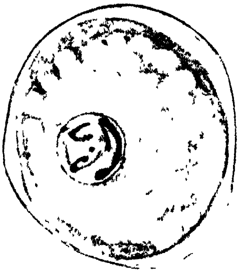
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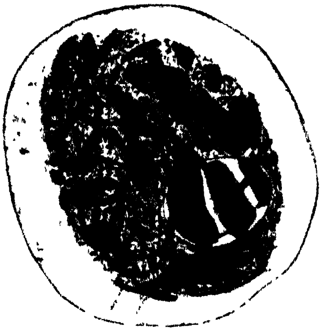
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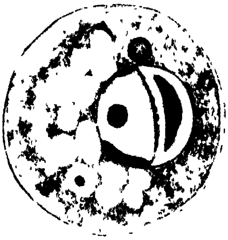
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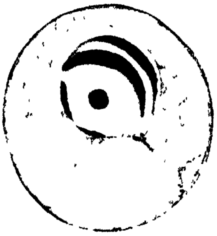
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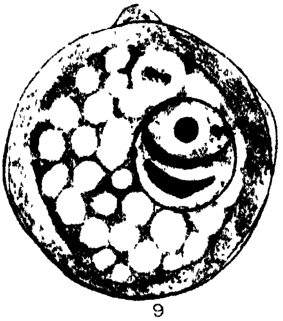
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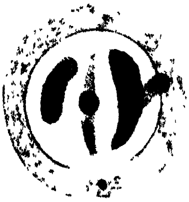
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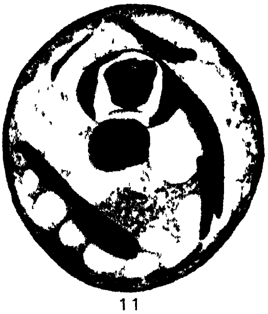
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PLATE 24

All figures of *Karyamoeba falcata* gen. nov., sp. nov., in mitosis. × 2500. Camera lucida drawings from faecal smears fixed in hot Schaudinn's fluid and stained in iron haematoxylin.

Fig. 12. Amoeba with clearly defined pellicle, hyaline zone, ectoplasm sharply delimited from the vacuolated endoplasm. No food bodies, but several siderophile granules in the endoplasm. Nucleus in the early prophase, with crescents transforming into polar caps, intermediate zone with stainable mass in which elongated, more deeply staining chromosomes are emerging. Small, dimly staining karyosome in the margin of this mass. Intradesmose with terminal centrioles each with halo forming in a meridional position but as yet reaching only to the tips of the polar caps.

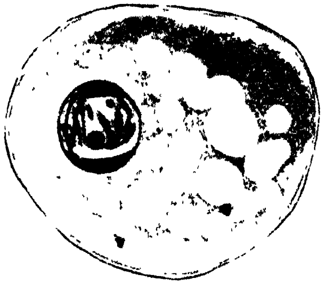
Fig. 13. Later stage. Centrioles near the poles, the upper one with halo visible. Chromosomes shortened.

Fig. 14. Later stage. Centrioles at poles, intradesmose curved. Chromosomes in equatorial plate in end-to-end arrangement. Persistent karyosome.

Fig. 15. Same stage. Nucleus in a vacuole. Halo around centrioles enlarged.

Fig. 16. Chromosomes scattered, perhaps an early anaphase. Note varying stainability of pseudopodium.

Fig. 17. Binucleate amoeba following nuclear division. Note absence of karyosome in one nucleus and its persistence in the other. One large crescent in each nucleus.



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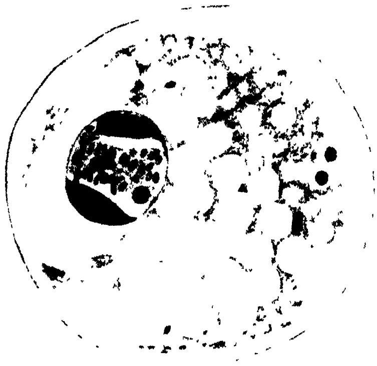

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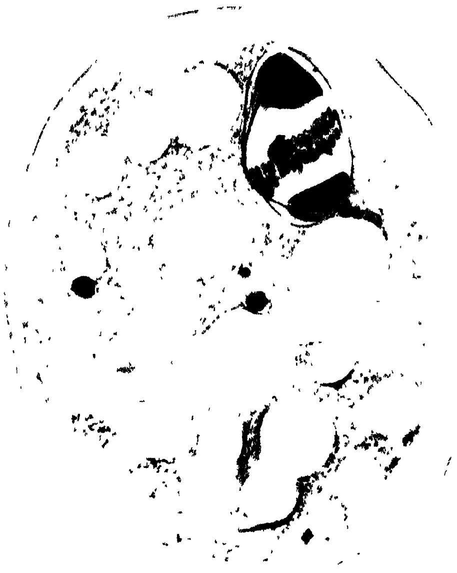

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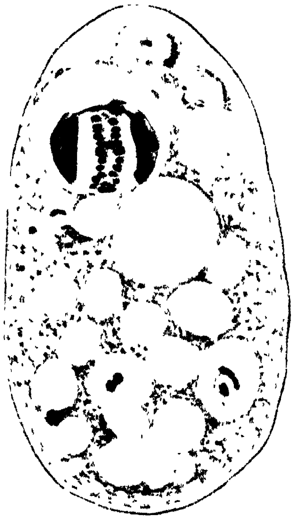

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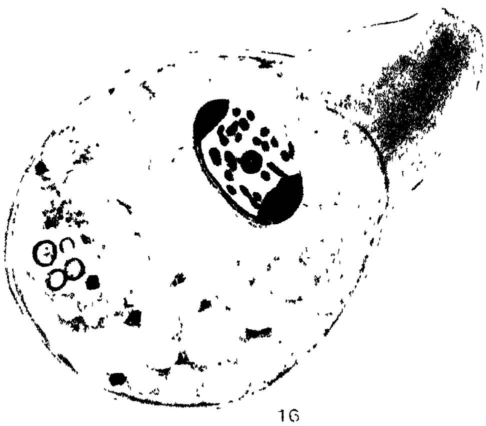

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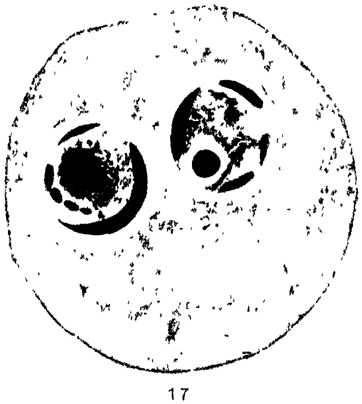

17

SURFACE CATCHES OF MARINE DIATOMS AND DINOFLAGELLATES MADE BY U.S.S. PIONEER BETWEEN SAN DIEGO AND SEATTLE IN 1923

BY

WINFRED EMORY ALLEN

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SURFACE CATCHES OF MARINE DIATOMS AND DINOFLAGELLATES MADE BY U. S. S. PIONEER BETWEEN SAN DIEGO AND SEATTLE IN 1923

BY

WINFRED EMORY ALLEN

(Contribution from Scripps Institution for Biological Research of the University of California) *

In the latter part of February, 1923, the United States Coast and Geodetic Survey Steamer *Pioneer* ended her winter work in the San Diego region and turned northward to prepare for summer work in Alaskan waters. On the northward trip, hourly collections were taken in the same way that they had been taken in the San Diego region (Allen, 1923). This series of collections was continuous to San Francisco. Collecting was resumed the first week in April and was again continuous as far as Seattle. It comprises a total of 119 catches from fifteen miles north of San Diego to a point near Seattle in Puget Sound.

On account of the uniform frequency of sampling in this series, it has seemed desirable to make somewhat different designations of regional units from those formerly used (Allen, 1922). Although arbitrarily selected, the limits of these regions have been largely determined by the more prominent coastal topographic features. (See map, fig. 1.) The position of the last catch in each region has been taken as the distal boundary of that region.

In seven out of the eleven regions designated, at least a few diatoms were recorded for every catch. Of dinoflagellates, this was true in only two regions. In five regions, the largest catch of diatoms, and in four regions the average number of diatoms to the catch, were greater than 50,000 to the liter. In only two regions did catches of dinoflagellates reach 1000 to the liter, and in no region was the average to the catch so great as 1000 to the liter. (See table 1.)

Fifty-one species of diatoms, representing twenty-two genera, were recorded between San Diego and Seattle. The more important of

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TABLE 1

To Show Gross Features of Regional Distribution

Region (see map)	Diatoms			Surface Temp. C.			Dinoflagellates		
	Largest catch*	Average per catch*	Number blank catches	Maximum catch	Average for region	Number catches	Largest catch*	Average per catch*	Number blank catches
Santa Catalina Island	32,627	6,122		13 89	14 00	12	110	40	2
Santa Barbara Channel	10,384	3,096		13 33	12 95	10	638	143	1
Piedras Blancas	3,164	368	2	12 22	12 00	13	22	3	11
Monterey	238,312	34,469		10 56	11.00	7	264	37	6
San Francisco	2,220,748	434,502		10 56	10 95	7	1958	693	
Point Arena	816,598	53,837	2	11.39	10 11	16	1246	136	8
Point St. George	594	157	2	9 44	9 56	13	594	2	12
Yakina Head	330	100	6	8 8	8 8	14	88	8	12
Columbia River	33,968	14,407		8 77	8 9	8	220	74	1
Destruction Island	682,017	444,507		8 60	8 60	9	801	231	
Puget Sound	595,104	159,030		7 60	7.8	10	356	131	2

* Numbers per liter.

these had already been observed in the catches made by McEwen and Mortensen in 1920 (Allen, 1922). In the main, the same forms tended to prominence, although *Nitzschia seriata* Cl. was much less conspicuous than in the catches of 1920. Only in the Santa Barbara region did this species attain a place in the list of five most abundant forms.

Eight genera represented by ten species were found to be among the five most numerous forms in one or more regions. Of these, the most prominent numerically was *Skeletonema costatum* (Grev), which occupied first place in four regions out of the six in which it was conspicuous. The genus *Chaetoceras* was second to *Skeletonema* only in numbers. In constancy of representation, it was, as usual, first. The specimens of this genus were mostly small and difficult to segregate, for which reasons the record is somewhat unsatisfactory. Small forms listed as *Chaetoceras* sp. were found in all regions. In addition, *Chaetoceras debile* Cl. (sometimes including *Ch. curvisetum* Cl.) and *Chaetoceras scolopendra* (Cl.) were notable in some regions. In this particular series, the genus *Chaetoceras* appears to be relatively much more prominent south of Monterey than in any region farther north.

The genus third in prominence in this series was *Thalassiosira*. Identification of the species was not fully satisfactory. Only two, *Thalassiosira baltica* (Grun) and *Th. gravida* Cl., were recorded although there may have been as many as four or five species present in some catches. This genus showed some prominence in the southerly regions, but it was so very abundant in the regions of Columbia River, Destruction Island, and Puget Sound as to suggest the idea that northerly conditions especially favor it.

Asterionella was more conspicuous than in most series and it was especially noticeable near San Francisco. Heretofore, only one species of this genus had been noticed, *Asterionella japonica* Cl., but in the course of study it became evident that another species, *Asterionella kariana* Grun, was also present. This species seems to be very fragile in many colonies, all the distal points of the cells being broken. This breakage probably led to more or less error in identification even after the presence of a second (and possibly a third) species was recognized, but it is very probable that *A. japonica* Cl. is, in this series, more prominent in the southerly regions, while *A. kariana* Grun is more prominent in the northerly regions.

Bacteriastrum, *Grammatophora*, and *Eucampia* are the other three genera which had representatives among the five most prominent forms in some regions, but there is nothing in the showing made by them which requires particular attention in this connection.

The catches of 1920 (Allen, 1922) did not give any strong indication that the species studied were peculiar to any particular section between San Diego and Puget Sound. It is therefore especially interesting to note that this more continuous series at a different season does give some evidence of restricted geographic range of some pelagic diatoms; e.g., the greater prominence of the genus *Chaetoceras* southerly, of *Skeletonema* medially, and of *Thalassiosira* northerly, and the possible difference in range of the two species of *Asterionella,* just mentioned.

As to geographic difference in quantity production, there are some interesting points. In 1920, the largest individual catches were made in the regions near to or north of Cape Mendocino. In this series, the largest catches were made in the San Francisco region, considerably south of that point (table 1 and fig. 1). Furthermore, the average production of catches in the San Francisco region is only slightly less than in the region of Destruction Island. On the other hand, only thirteen out of the sixty-five catches south of Cape Mendocino contained more than 10,000 to the liter while thirty-three of the fifty-four catches north of Cape Mendocino exceeded that number. Superficially, this seems to be strong indication that productivity is greater in higher latitudes. It is, however, one thing to say that higher latitudes are places of greater production and quite another thing to say that they cause greater production in the sea. For example, they may merely mark the region in which land drainage most favorably influences marine productivity. The showing in the San Francisco region makes it desirable to delay the statement of a conclusion until we are sure that latitude exerts a determining influence instead of merely accompanying such an influence. (Allen, 1922, p. 143.)

Although it was not possible to make hydrographic observations to accompany this series of collections, records of temperatures at times of collecting were regularly made. These temperature records are partly summarized in the middle columns of table 1. As might be expected, this summary shows a steady lowering of temperatures toward the north. Six out of eleven maximum catches were taken in temperatures slightly below the average for the region, but there is no special significance in this isolated fact. The fact that the two most consistently productive regions were those of lowest temperature while that of largest production was of a somewhat higher temperature is important because it adds to the evidence that, under ordinary circumstances, temperature is not a directly determining factor in productivity of marine diatoms.

In considering the evidence of productivity in this series of catches, it should be remembered that the southerly regions were traversed in late February (not usually a highly productive month), while the northerly regions were traversed in April (in some years the most productive month). For that reason, direct comparison of areas south of San Francisco with areas north of that point is rather unsatisfactory. So far as we really know, the southerly sections may also have been heavily productive in April. Still, it is not probable that the relative conditions of temperature were very different in the two months, since it is known that southerly sections get some of their coldest water in March and early April.

At this time of year, the greatest change in any single environmental condition is supposed to be in the effect of the sun's rays. There are excellent general and theoretic reasons for connecting vernal increase in productivity with solar influence, but, unfortunately, that is one factor concerning which marine researches are almost entirely lacking.

Comparison of productivity of regions in this series with that of those in the 1920 series shows that the Destruction Island and San Francisco regions were highly productive in all three cases (McEwen in summer, Mortensen through fall, and the *Pioneer* in early spring). The Point St. George region was poorly productive in all cases while the Yakina Head region was most productive in Mortensen's catches and very poor in McEwen's and those of the *Pioneer*. Thus we find additional evidence that some regions may be highly variable in productivity according to season or circumstance. On the other hand, it seems probable that some regions, e.g., Destruction Island, may always show relatively high productivity as compared with other regions.

It is not possible at present to make a conclusive statement essentially different from that concerning the 1920 series (Allen, 1922). This is, however, an appropriate place to emphasize the fact that, with such vessels as the *Pioneer*, plankton collecting of very high value can be done, and that the spirit of coöperation shown by the officers and men of the U.S.S. *Pioneer* is exceedingly encouraging for more rapid and effective advances in oceanic research. The enthusiasm of the administrative officers of the U. S. Coast and Geodetic Survey in Washington is also encouraging and deserving of high commendation.

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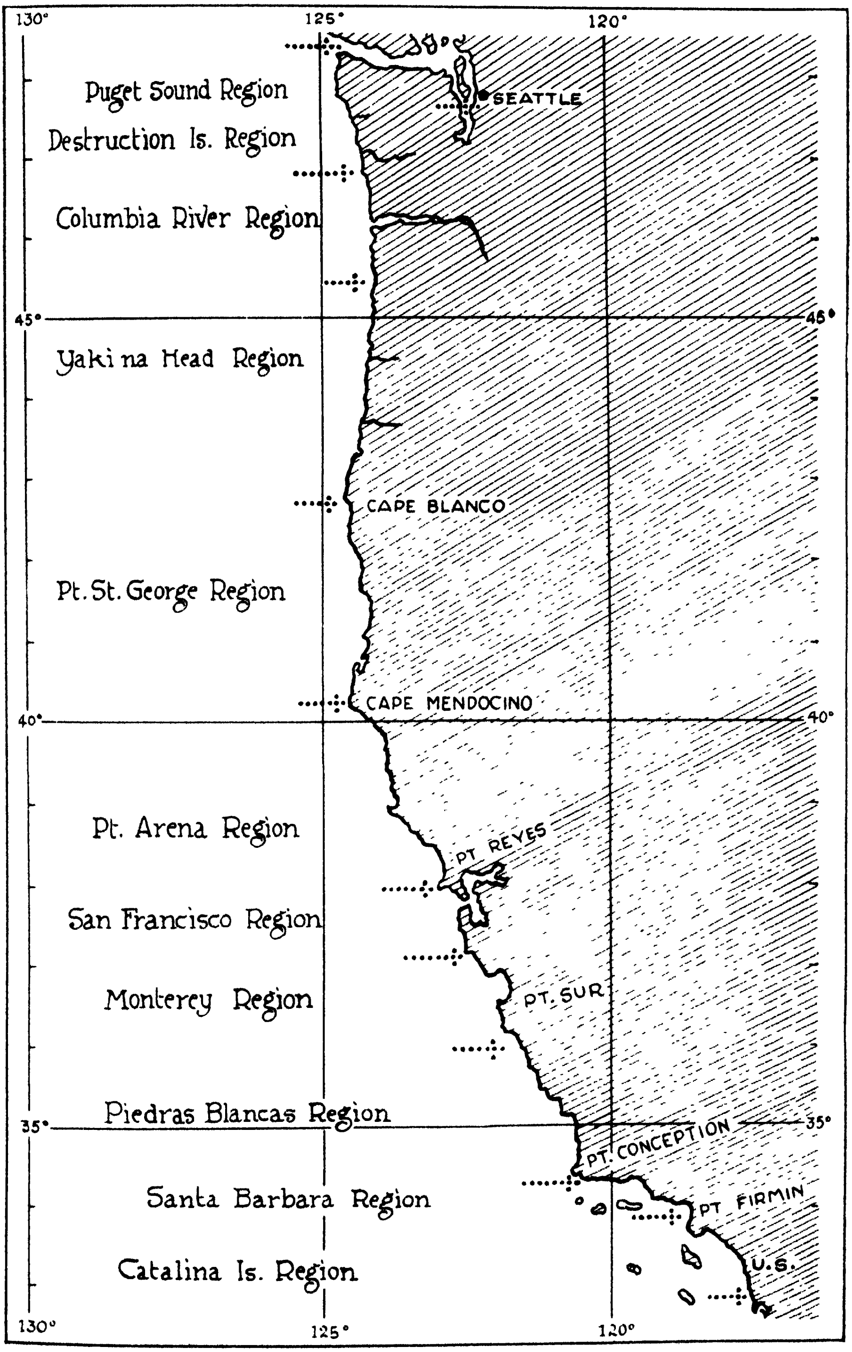

Fig. 1. Map Diagram of Pacific Coast of United States. Regional Boundaries indicated by dotted lines.

VIABILITY OF CYSTS OF HUMAN INTESTINAL AMOEBAS AS DETERMINED BY EXPOSURE TO VARIOUS SUBSTANCES AND SUBSEQUENT STAINING IN HAEMATOXYLIN

BY

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VIABILITY OF CYSTS OF HUMAN INTESTINAL AMOEBAS AS DETERMINED BY EXPOSURE TO VARIOUS SUBSTANCES AND SUBSEQUENT STAINING IN HAEMATOXYLIN

BY

NATHANIEL BERCOVITZ, M.D.

INTRODUCTION

The following study was suggested by two facts. During a series of experiments to study the reaction of the cysts of human intestinal Protozoa to stains, it was found that cysts of *Councilmania lafleuri* Kofoid and Swezy took the Congo red stain when brought in contact with an aqueous solution of that stain, while, in many cases, cysts of *Endamoeba coli* did not take the stain.

During the experiments (reported in Bercovitz, 1923), one specimen was found to contain cysts of both *Councilmania* and *Endamoeba coli;* and some of these cysts, after contact with Congo red, took the stain while others did not. It naturally was of interest to find out whether or not the cysts taking the stain were from *Councilmania,* and whether or not those which did not take the stain were from *Endamoeba coli.* An attempt was accordingly made to locate a number of cysts on slides, some stained with Congo red, and some not stained. These smears were then stained in haematoxylin so that the cysts might be differentiated by their nuclear structures.

The cysts were located, and the slides stained in the usual manner, but when it came to examining the slides a most peculiar phenomenon was observed: the cysts exposed to Congo red did not stain at all except for a deposit of stain on the cyst wall. Since there might have been some mistake in the technique, the tests were repeated, but with the same result. In all, eighteen slides were stained, and in each case the cysts showed an abnormal type of staining with haematoxylin after they had come in contact with the Congo red. In both species, cysts from the same specimens not in contact with the Congo red stained as usual with haematoxylin, and showed in a typical manner the characteristic structure of cytoplasm and nuclei.

Hence it was concluded that contact with the Congo red was responsible in some way for this change in the staining reaction; and while there was disappointment that the cysts in this instance were not differentiated, there seemed to be in this phenomenon possibilities of interesting results from a study of intestinal parasites following treatment with various substances.

The second matter which brought about this study was the reaction of cysts when treated with various disinfecting chemicals. The writer had returned to America after six years spent in a mission hospital in tropical China, and the investigation then interesting him was the use of the septic tank as a means of solving the sanitary—as well as economic—problem of China with respect to the use of human fertilizer. The idea was this: Faeces after treatment in a septic tank still retain their value as fertilizer. If the treatment might be so modified that it would also destroy in the faeces the cysts of intestinal parasites, then the problem would be solved, since the capacity of the parasites to disseminate certain parasitic diseases would be destroyed and the use of night soil as fertilizer might thus be safely continued.

The cysts proved remarkably resistant to a number of agencies, and so the study was concentrated on the effect upon the cysts of a variety of disinfectants. The action of an aqueous solution of eosin was taken as the criterion of death, those cysts which took the stain being considered dead, and those which did not stain being considered alive (Boeck, 1921). Staining in eosin solution, it might be noted here, is a reliable criterion of death if the cysts have been killed by heat.

The author had demonstrated in previous work that chlorinated lime in sealed glass ampoules would retain its full strength for over three years. This substance, moreover, is a valuable disinfectant. For these reasons it was hoped that chlorinated lime might be advantageously used to destroy cysts of intestinal parasites; it was accordingly tried first of all. But the cysts of human intestinal parasites were not injured by the chlorinated lime.

The test, however, seemed to result in an undue proportion of living cysts, even after treatment with rather strong solutions of powerful antiseptics. For instance, cysts immersed in a one per cent solution of bichloride of mercury for twenty-four hours did not, generally, take the eosin stain; and this was found to be true with other chemicals. At this point the experience of previous experiments was recalled, in which some cysts of *Councilmania* and of *Endamoeba* *coli*, after exposure to Congo red, could not be stained with haematoxylin, and it was decided to use haematoxylin staining as a test of the efficacy of treatment with a number of the common disinfectants. Cysts which have been treated before staining in hematoxylin show coagulation of the cytoplasm, and their nuclei either fuse with the cytoplasm or appear as black spores therein. Neither cytoplasm nor nuclei show the characteristics of live cysts. These results make it clear that this method of staining cysts in haematoxylin after treatment with certain chemicals and disinfecting substances opens a new field in the study of the life processes of these organisms.

The work thus begun is far from complete. The writer cannot continue it because of his return to China, therefore the results so far achieved are here presented in the hope that their publication may stimulate further investigation along this line.

MATERIAL AND METHODS

The preliminary work was done with a number of the chemicals used in the laboratory for disinfecting purposes, in varying strengths, and on a number of types of cysts. In each case the cysts so treated did not stain normally with haematoxylin, and systematic study of the problem was begun.

Eleven substances were used in this study, viz.: (1) 1 per cent solution of hydrochloric acid; (2) 1 per cent solution of bichloride of mercury; (3) 1 per cent solution of carbolic acid; (4) 1 per cent solution of formalin; (5) 70 per cent alcohol; (6) 1 per cent aqueous solution of eosin; (7) 1 per cent solution of lysol; (8) 1 per cent solution of sodium hydroxide; (9) 1 per cent solution of chlorinated lime; (10) 1 per cent acetic acid; and (11) brine, half strength.

Because of their large size it was decided to use the two types of eight-nucleated cysts, *Endamoeba coli* and *Councilmania lafleuri*. Specimens containing large numbers of these cysts were obtained, and the tests were made according to the following plan.

A drop or two of the chemical was placed in a vial, and a small amount of the faeces containing the cysts was thoroughly rubbed up in this fluid; smears were made immediately and at intervals of fifteen minutes and one hour thereafter. They were fixed in Schaudinn's fluid heated to 60° C. In this way we hoped to be able to determine whether or not disinfectants as commonly used have an immediate effect on cysts; and, if not, how much time elapses before any effect can be noted. The importance of this can be readily appreciated by those who work in laboratories and hospitals, where hands and utensils must be disinfected after the use of infected material. This procedure might also be expected to throw some light on the length of time of exposure to disinfectants required to kill cysts in faeces, both in hospital practice, and in disinfecting night soil, especially in times of epidemics.

ACKNOWLEDGMENTS

Acknowledgments are made to the China Medical Board of the Rockefeller Foundation for the opportunities offered for this study through a traveling fellowship; to the University of California for a technical assistant compensated by a grant from the Research Board; to the California State Board of Health for access to material; and to Professor Charles A. Kofoid and Dr. Olive Swezy for advice and counsel.

DISCUSSION OF POSSIBLE ERRORS

In this work possible sources of error are: (1) Failure to secure adequate suspension of material so that all cysts come in contact with the chemical equally. Failure to do this may account for an occasional cyst staining normally in a slide containing large numbers of cysts staining abnormally. (2) Incomplete destaining in iron alum; (3) Difficulty of describing and interpreting what was seen, which will become evident as the results are noted.

The problem of destaining was a difficult one. In many cases it seemed almost impossible to destain at all. The whole series of slides was re-destained after first observations were made, the second time for double the length of time customarily used in normal staining.

In describing the cysts which were treated the difficulty has been to determine in every case whether the cyst wall only had taken the stain, or whether the protoplasmic contents also had become so modified by the action of the chemicals used as to take a dark, uniform stain throughout, with no differentiation in nucleus or cytoplasm. This second result is referred to in our discussion as abnormal type of staining. Under ordinary conditions, in untreated material the cyst wall does not stain, and only certain moribund cysts show a dark uniform stain that resists all attempts at differentiation.

In some cases there seemed to be an outer shell which was clear and very delicate, and which seemed to be detached from the inner shell which was adherent to the cytoplasm. Between these outer and inner layers was a perfectly clear zone. Stained débris was never seen within this clear zone, and it was assumed that the stain was entirely within the cyst wall. Possibly the apparent detachment was due to the shrinkage of the contents of the cyst because of the action of the chemical. In most cases where this clear zone was seen, the cyst, apparently, was densely stained, either in the inner layer of the wall, or evenly throughout.

When the contents of the cyst did not stain, it was probably due (1) to the chemical itself filling the cyst wall, and thus preventing the penetration of the stain; (2) to some chemical reaction with the cyst wall, such as coagulation, or (3) to some other change which prevented or hindered the passage of the mordant, stain, and decolorizer, or one or more of these reagents.

Perhaps the most important observation made was that the chemicals used produced definite changes in the cyst which may be interpreted as lethal changes. Were it possible to culture these cysts, their viability or lack of it would be definitely established. But, from the morphological viewpoint, these chemicals seem to bring about changes in the cysts which appear to be incompatible with life. Departures from normal reactions to stains are often observed in moribund cells.

If this is the case, then the eosin test as specific for determining the viability of cysts must be questioned. For these cysts are apparently dead, yet they do not take the eosin stain as dead cysts are supposed to do. It may be, therefore, that a new criterion of viability of cysts must be found. Until viability can be definitely established or disproved by culture experiments, the writer submits the staining of cysts and the study of their morphology as a means of determining their apparent viability or death.

In the hospital in Kachek the writer is continuing these experiments with ova of the hookworm. From the chemical standpoint, there seems to be an analogy between the cyst walls and the shell of the egg of a hookworm. It has been impossible so far to determine the chemical composition of cyst walls, and that has been a drawback to the accuracy of the experiments. But in the tropical climate of Kachek, the development of the larva within the egg of the hookworm can be observed until the larva emerges, and thus the study of reagents which will kill the hookworm egg is simplified. The results of these experiments will be the subject of another communication.

EFFECTS OF SUBSTANCES USED ON SUBSEQUENT STAINING OF CYSTS WITH HAEMATOXYLIN

In the reports of tests which follow, *a*, *b*, and *c* designate exposures to reagents of 15 seconds, 15 minutes and 1 hour, respectively.

1. Hydrochloric Acid 1%

Endamoeba coli.

a. In some cysts there was shrinkage from outer cyst wall which showed as a clear membrane; some cysts showed nuclei staining very faintly, and in others nuclei appeared as light unstained areas in the darker background of cytoplasm; cytoplasm appeared coagulated with more or less vacuolation. No normal cysts seen.

b. No nuclei seen; some cysts showed irregular staining of cytoplasm; some black after prolonged destaining, hence dead, or showing considerable modification in structure of cytoplasm.

c. Some cysts showed a uniform dark stain; others, nuclei as ghosts in black background; color held very fast after prolonged destaining.

Councilmania lafleuri.

a. Few cysts almost normal; others showed chromatin of nuclei as black clumps; cytoplasm irregularly stained, and somewhat granular; cysts decidedly not normal.

b. Several cysts stained homogeneously with no nuclei showing; coarse granules on cyst wall; cytoplasm probably greatly changed.

c. Impossible to destain cysts; no distinct membrane; cytoplasm granular; nuclei appeared as black clumps; cysts somewhat shrunken; none normal as to nuclei, cytoplasm, or membrane, and all probably dead.

Comment.—Destructive changes in the cysts appear to be present with brief exposure of 15 minutes or even less. The action of the gastric juices on cysts should be investigated with respect to the destruction of the cysts by the acid in the juices.

2. Bichloride of Mercury 1%

Endamoeba coli.

a. Various effects: stain abnormal; some penetration of stain with nuclei faintly staining; apparent coagulation of cytoplasm; very even staining of cyst wall, with no structure of cytoplasm evident, and, in some, ghosts of nuclei are seen through the cyst wall.

b. Many cysts showed no penetration or abnormal type of staining, some with collapse of cyst wall. In a few, nuclei showed as black masses at one end of cyst. In some cytoplasm appears granular.

c. Shell of cyst stained, no nuclei or cytoplasm could be differentiated. Some cysts collapsed.

Councilmania lafleuri.

a. Most cysts showed no penetration or an abnormal type of stain; some a slight penetration of stain, with reticulated cytoplasm, but no nuclei seen.

b. Cysts showed stained; some seemed to be disintegrating. Some cysts seemed fibrous after prolonged destaining. No nuclei seen.

c. All cysts more or less irregular, and in some collapse complete. In few cysts cytoplasm seemed to be stained, but apparently as part of destructive action of chemical. All cysts in some stage of disintegration; none seemed at all normal.

Comment.—Here the possibilities were precipitation of the chemical on the wall of the cyst, or coagulation and destruction of wall of cyst by the chemical. Morphologically, no cysts appeared normal after exposure to the bichloride of mercury. If, however, the cyst wall merely retained the chemical, and prevented its entrance into the cyst, the cyst might, at least after very short exposure, still have been alive. However, this hardly seems possible.

3. CARBOLIC ACID

Endamoeba coli.

a. Many cysts showed no penetration or an abnormal type of stain; some cysts shrunken. Other cysts showed destruction beginning, with slight penetration of stain, and nuclei faintly seen.

b. Cysts more or less distorted, and very dark even after prolonged destaining. In some, nuclei appeared as black masses in the dark cytoplasm. Some cysts showed a dark uniform stain.

c. Heavy, homogeneous staining, with no differentiation of parts of cyst, possibly an even staining of cyst wall. In one cyst, wall stained, and cytoplasm black mass at one end.

Councilmania lafleuri.

a. Very even staining of cyst, either of wall only or of cytoplasm with imperfect fixation. Cysts more or less shrunken.

b. Results here same as for exposure for 15 seconds.

c. Cysts small, shrunken, dark even after prolonged destaining, and in a few cases black masses indicated nuclei. Probably all cysts were dead.

Comment.—These cysts appeared to be vitally affected by the carbolic acid, and on morphological evidence would have been considered dead. After exposure to carbolic acid for an hour most of these cysts showed very little penetration of eosin dye. Cysts that had been exposed to bichloride of mercury gave the same reaction. Is eosin, therefore, a reliable criterion of the viability of the cysts?

4. FORMALIN 1%

Endamoeba coli.

a. Abnormal type of staining.

b. Cysts separated from other material on slide by clear zone; cysts appear shrunken. Irregular staining, probably of surface only, as no nuclei seen except in two cases where they stained very faintly.

c. Cysts uniformly shrunken and irregular. No nuclei seen, and staining seemed to be limited to wall of cyst.

Councilmania lafleuri.

a. Cysts stained very slightly, but no nuclei seen.

b. Cysts separated from material on slide by a clear zone which seemed to be a very thin membrane (outer layer of cyst wall?). Irregular staining, apparently of cyst walls only, as no nuclei seen, or differentiation of cytoplasm.

c. Cysts shrunken and heavily stained, but no signs of nuclei. Retraction of inside mass from outer clear zone very marked.

Comment.—These results recall experiments with ova of *Ascaris lumbricoides* immersed in formalin for many years without impairment of the viability of the ova. While the cysts in this series of experiments are, by morphological standards, dead, it would be interesting to note the effect upon them of culture treatment after immersion in the formalin.

5. ALCOHOL 70%

Endamoeba coli.

a. Cysts remained black even after prolonged destaining. Slight shrinkage of cyst noted in most cases, and very even distribution of stain. In one cyst ghosts of nuclei seen.

b. A few black cysts found.

c. A number of cysts found, all very heavily stained, and all slightly shrunken away from a very thin membrane which separated cyst from surrounding material by a clear zone.

Councilmania lafleuri.

a. Heavy, even staining of cysts without any differentiation of cytoplasm; no nuclei seen.

b. Heavy, even staining of cysts, which were slightly irregular in outline. Two particularly well destained cysts showed six and eight black clumps, respectively, probably nuclei. These two cysts may have been dead before the treatment, as all the other cysts seen were black and homogeneous.

c. Cysts were shrunken, and stained rather irregularly. Degenerative changes noted, and in no cases were nuclei seen.

Comment.—The cysts appeared to be dead after prolonged treatment with alcohol, so far as could be determined from their morphology.

6. EOSIN 1%

Endamoeba coli.

a. Some cysts stained normally, others showed abnormal type of staining. Majority of cysts seen very dark in one segment, and smaller than normal.

b. After prolonged destaining, some cysts showed slight staining of cytoplasm, and nuclei were seen. Many cysts showed abnormal type of staining.

c. All cysts showed an abnormal type of staining.

Councilmania lafleuri.

a. Cyst wall apparently slightly stained. Slight shrinkage of cysts. No nuclei seen in any of cysts except one.

b. Many cysts seen. All seemed about normal in size, and all showed staining of wall, but no evidence that stain had penetrated to cytoplasm.

c. All cysts showed the abnormal type of staining.

Comment.—The point of interest and importance here is that there was an apparent blocking of the passage of the stain through the cyst wall even when an aqueous solution was used on normal cysts. The penetration of eosin was used as the criterion of death (see p. 0), and it is very evident that there is an apparent abnormal modification of the stain in these normal cysts when they are exposed to the eosin. As has already been noted, there is quick penetration of the eosin after the cysts have been exposed to heat, but this is not the case after the cysts have been exposed to other substances which produce, morphologically, changes which would seem to be incompatible with the continued life of the cyst.

7. LYSOL 1%

Endamoeba coli.

a. Cytoplasm of cysts apparently coagulated, and in a few cases a few black clumps, probably nuclei, seen.

b. Cysts showed coagulation, and some vacuolization, but no traces of nuclei seen.

c. All cysts seemed dead, being irregular in outline, even to point of being apparently ruptured, with degenerative changes in cytoplasm.

Councilmania lafleuri.

a. Cysts heavily and irregularly stained. In one, two clumps, probably nuclei, seen. In another cyst, several black clumps seen.

b. Cysts very heavily stained. In most cases cyst wall collapsed. No differentiation of cytoplasm or nuclei noted. Apparent coagulation of cytoplasm.

c. Color apparently held in shell, heavier at one pole of the cyst than the other. Collapse of cyst wall in some cases, but in seventeen cysts no differentiation of cytoplasm or of nuclei observed and cysts appeared to be killed.

Comment.—From the evidence, there seems to be no doubt that lysol killed the cysts, and very quickly too. This becomes of special interest and importance in a study of the viability of the cysts after exposure to disinfecting materials and of criterions of death when the following comparisons are made. In *Endamoeba coli*, after exposure to 1 per cent lysol solution for an hour, only 30 per cent of the cysts stained with eosin. In *Endamoeba dysenteriae,* after exposure to 1 per cent lysol solution for forty minutes, 100 per cent of the cysts stained with eosin. And yet cysts of *Endamoeba coli*, when stained in haematoxylin after exposure to lysol solution, appeared as killed cysts. So, while the eosin test seems to be reliable in the case of *Endamoeba dysenteriae* after exposure to lysol, it does not seem to be reliable in the case of *Endamoeba coli.*

8. SODIUM HYDROXIDE 1%

Endamoeba coli.

a. Cysts slightly swollen, there being only a very small space between the so-called outer and inner membranes. Cysts faintly stained in a few cases, with nuclei faintly seen. In most cases staining is even, and abnormal in type.

b. A few cysts stained normally except that they were swollen.

c. Cysts appeared to be dead. All more or less swollen, and cytoplasm rather unevenly stained. Occasionally cysts showed small, black, atypical clumps, probably degenerating nuclei.

Councilmania lafleuri.

a. In all cysts seen some swelling apparent. Cytoplasm granular, and some atypical nuclei seen. Majority of cysts seem to show evidences of degeneration.

b. A number of cysts seen irregular in outline, and somewhat mottled, with nuclei showing as black clumps. In other cysts apparent coagulation observed, and in one or two abnormal staining.

c. Cysts swollen and irregular. Some apparently took no stain, others showed staining of cytoplasm, others held stain very tenaciously, and still others showed apparent coagulation of cytoplasm. In only a very few cases, evidence of nuclei.

Comment.—The swelling of cysts in sodium hydroxide has been noted in other experiments by the writer. Subsequent exposure to eosin after treatment with sodium hydroxide showed indistinct staining: the edges of the cysts were not clean cut. Apparently the cysts treated seemed to react differently when stained. The effect of alkaline duodenal juice on the cyst ought to be an interesting study with respect to viability of the cyst.

9. CHLORINATED LIME 1%

Endamoeba coli.

a. Cysts showed abnormal type of staining.

b. Some cysts showed heavy, even staining. In a few cases, cysts destroyed. Some cysts showed nuclei faintly stained as clumps.

c. Cysts exceedingly difficult to destain. All seemed to be dead.

Councilmania lafleuri.

a. A number of cysts showed penetration of stain with outline of nuclei showing. In some cysts, apparent coagulation of cytoplasm, in others, abnormal type of staining.

b. After prolonged destaining majority of cysts seen intensely stained, no nuclei showing. Cysts probably killed.

c. Apparent homogeneous mass of color either on wall of cyst, or else whole cyst stained, very intense, with no differentiation of cytoplasm or nuclei.

Comment.—The effect of chlorinated lime on these cysts was noted with particular interest because of previous experience with the use of eosin after exposure of cysts to chlorinated lime (Bercovitz, 1922), in which the cysts did not take the eosin, and so, according to that criterion, should be considered as still viable. The appearance of these cysts, however, when stained with haematoxylin after exposure to chlorinated lime is that of dead cysts.

The importance of this observation cannot be overestimated when the usefulness of chlorinated lime as a general disinfectant is remembered. If the eosin test is to be the criterion of viability, then chlorinated lime cannot safely be used as a disinfectant. If, however, the appearance of cysts stained in haematoxylin after exposure to chlorinated lime is to be considered, more than a reasonable doubt exists as to their being alive.

10. ACETIC ACID 1%

Endamoeba coli.

a. Shape of cysts markedly altered in all cases, most of them being shrunken, and several apparently destroyed. In a few, stain abnormal, in a few others a fine reticular cytoplasm; but nuclei seen in only one cyst.

b. The same results noted after 15 minutes as after 15 seconds, except that in none of the cysts any signs of nuclei seen.

c. Cysts greatly shrunken, and stained very heavily. No differentiation of cytoplasm or nuclei. Probably, the cysts being dead or contents greatly modified, all portions stained evenly.

Councilmania lafleuri.

a. Much débris around majority of cysts; no evidence of penetration. A few cysts destroyed.

b. One cyst showed outline of nuclei, others intense staining, with some shrinkage, and much débris around cyst.

c. Condition of cysts after one hour the same as after 15 minutes.

Comment.—In general, the shrinkage of cysts of *Endamoeba coli* was greater than that of the cysts of *Councilmania*, but in no case did any of the cysts appear as normal, live cysts.

11. Brine, Half Strength

Endamoeba coli.

a. Cysts showed smooth, even staining, probably of surface of cyst, black, in a very light field, after prolonged destaining. No nuclei seen. Cysts slightly distorted.

b. Cysts heavily stained, and shrunken, but no nuclei seen.

c. Cysts distorted, and abnormal, as in exposure of 15 minutes.

Councilmania lafleuri.

a. Cysts irregularly stained, very black with no differentiation of structure. In one cyst black clumps at one pole were evidently nuclei. Cysts more or less shrunken.

b. Majority of cysts collapsed. Outer membrane irregular. No nuclei seen, and cysts probably all dead.

c. Majority of cysts destroyed. In one, nuclei stained, stain probably penetrated after cyst was killed. Majority of cysts collapsed and in various stages of destruction.

SUMMARY

1. Staining with haematoxylin after treatment with various chemicals and disinfectants is of more value in the determination of viability of cysts of human intestinal amoebas than the present staining with eosin.

2. Cysts of *Endamoeba coli* and of *Councilmania lafleuri* are killed, if morphology be any guide, by treatment with bichloride of mercury, carbolic acid, lysol, or formalin, all in strength of 1 per cent or with brine half strength, after exposure for a maximum period of one hour. This conclusion should be verified or disproved by culture experiments.

Transmitted April 15, 1924.
Kachek, Hainan, China.

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University of California,
Berkeley, California.

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THE INCOMPLETE DIGESTIVE TRACT OF APPENDICULARIA SICULA

BY

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THE INCOMPLETE DIGESTIVE TRACT OF APPENDICULARIA SICULA

BY

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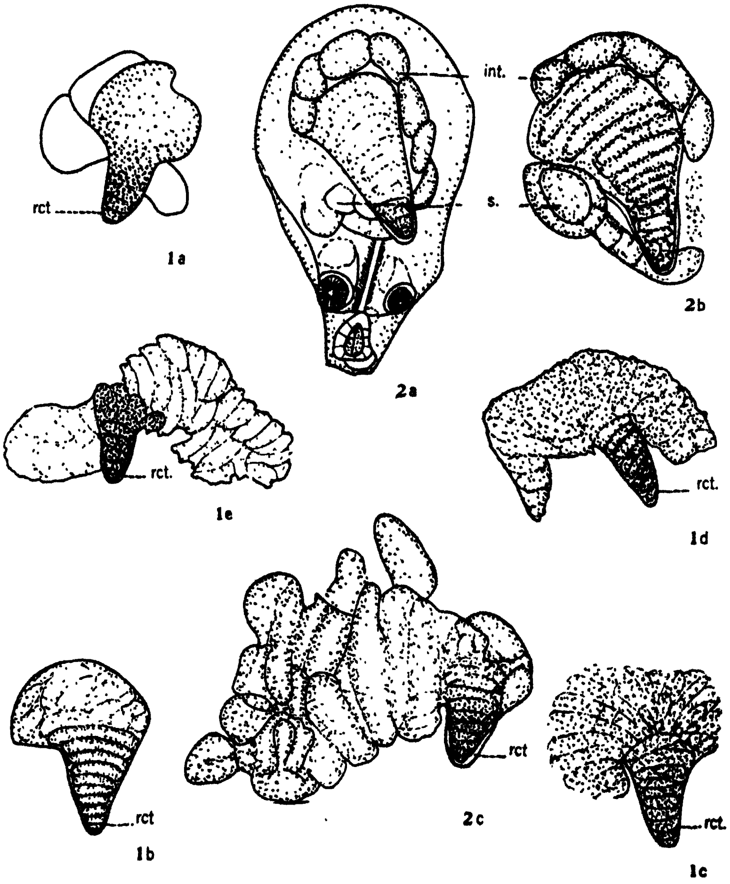
The Copelata fauna in the waters of La Jolla, California, has been more meager this winter (1923–1924) than any previous winter in my recollection. The causes—lack of rains, lack of storms, etc.—which may have affected the change in distribution of those tunicates we do not know. But the fact remains that *Fritillaria,* so abundant during previous winters, has been only rarely found this winter, and then in very limited numbers, both as to individuals and species.

Appendicularia sicula, however, usually scarce and irregular in this vicinity, occurred more regularly and in greater numbers during the months of November and December, 1923, than during any of the previous years on record. In each morning's catch 2 to 10 specimens were found, so that a sufficient supply of living material was available to permit of observations and some experimental work; and the author was thus enabled to remove some doubt which existed in her mind as to whether or not the huge rectum of this tunicate is a blind sac without any anal opening.

Appendicularia sicula is unique in its relation to other Copelata. Its general appearance and the absence of a hood mark it as closely related to *Oikopleura,* although it is smaller than the smallest species of that genus, the length of its trunk being only .3 to .4 mm. In the shape of the house, the very small stomach, and the hepatic tissue of the rectum it resembles *Kowalevskia* more closely than any other genus; while in respect to the shape of its pharynx and tail, it belongs in the *Fritillaria* group. But the most conspicuous organ, characteristic of this genus only, is the huge, pear-shaped rectum, which is always loaded with a dark brown substance. The older the animal, the more expanded and closely packed is its rectum, covering and obscuring all other organs in the vicinity.

It has always seemed strange that the huge rectum of *Appendicularia sicula* should be constantly crammed full, and yet not the slightest evidence appear of a discharge of the contents through the anus, as is frequently observed to happen in other Copelata, especially when a slight pressure is applied to the animal. This winter, while *Appendicularia sicula* were so abundant, living animals under the microscope were watched for hours, but never was there discovered an anal aperture, nor any discharge of fecal matter. When a slight pressure with a cover-glass was applied to the animal, the pressure being gradually increased on the supposition that, if there was an anal opening,



Figs. 1–2. Successive changes of the rectum of *Appendicularia sicula*. Abbreviations: *int.*, intestine; *rect.*, lower end of rectum; *s.*, stomach.

Figs. 1*a*–1*e*. Changes of rectum after gradual increase of pressure. × 80.

Fig. 2*a*. Ventral view of trunk showing rectum in normal condition.

Figs. 2*b*–2*c*. Rectum of the same trunk after applied pressure. × 80.

some of the contents of the rectum would be forced to escape through it, it invariably gave the results shown in the series of illustrations (figs. 1*a*–1*e*, and figs. 2*a*–2*c*). Upon the application of a slight pressure the rectum expanded. Increasing the pressure gradually caused it to expand more and more until finally the walls broke at the upper broad end, letting the contents escape from that point; while the lower, narrow end, where, according to Fol (1874), the anus is situated, remained intact, surrounded by a very strong, thick membrane in which no trace of any opening was to be found.

These observations and experiments on numerous animals led to the conclusion that the rectum in this copclate differs from that of any other, not only in its appearance and enormous size but also in its function. The animal does not discharge the waste matter through an anal opening but retains it in the rectum, which seems to serve as a storage place where the food substance, after leaving the stomach, is packed in coils, to remain there until the animal dies. This explains the increase in size of the rectum and the bulk of its contents in older animals. These coils hold together so well that they can resist a comparatively strong pressure without breaking. They are so solidly packed that in older animals the rectum appears quite black, and in fact only the black speck of the transparent animals is seen as they move swiftly with their characteristic jerky motion.

It is with some hesitation that the author ventures to contradict the statement of such an able investigator as Fol (1874), who has described the genus in great detail, and who places the anus, both in his description and in his illustration, at the lower, narrow end of the rectum and a little to one side. Lohmann (1896) does not give any detailed description but refers to Fol's work. Examination of numerous *Appendicularia sicula,* both dead and alive, under high pressure and in normal condition, and from every possible angle, invariably revealed the lower, narrow end of the rectum covered by a continuous, heavy membrane, with no rupture or opening whatever. The statements therefore seem fully justified: (1) that there is no anal opening in *Appendicularia sicula,* and (2) that the huge rectum of the animal is a blind sac which serves as a place for the storage of food remnants.

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EXPERIMENTAL AMOEBIASIS IN CATS FROM ACUTE AND CHRONIC HUMAN CASES

BY

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INTRODUCTORY AND HISTORICAL

The literature shows that the experimental infection of cats by the rectal injection of motile *Endamoeba dysenteriae* has been attempted with dysenteric stools obtained from acute human cases of amoebiasis, rather than with cysts from chronic cases. Due to the fact that the differentiation of the species of the amoeba causing dysentery in man was not fully established until 1903 a review of the experimental work prior to that date by Lösch (1875) and other early investigators will be omitted here. Craig (1911) states that in one instance he gave a kitten a rectal injection of 5 c.c. of faeces from a case of dysentery. In Wenyon's (1912) experimental infection of kittens by rectal injections, a specimen was used which contained a small amount of blood, mucus, and amoebas. Darling (1913) states that

> if kittens be inoculated rectally with material from an adolescent or *histolytica*-like strain, the strain may be carried upon the death of the preceding kitten for four, five, or six transfers, . . . but if, on the other hand, a chromidia containing senile "*tetragena*" like strain be injected rectally into kittens, it cannot be carried through more than one or two transfers. Indeed, it may perish in the first kitten inoculated.

Baetjer and Sellards (1914*b*) successfully inoculated kittens with nine strains, six of which were obtained from patients with symptoms of varying intensity. The remaining three were atypical strains from individuals suffering from diarrhoea characterized by watery stools which, in all but one case, were free from blood and mucus. The authenticity of the atypical strains has been questioned by Dobell (1919). No statement is made by Izar (1914) as to whether the faeces which he used for inoculation of kittens were dysenteric or not, but this was presumably so, as the larger part of his discussion concerns human amoebic dysentery. More recently Dale and Dobell (1918) carried a strain through forty-three passages in 106 kittens. They obtained this strain from a case of recurrent dysentery of subacute type and the specimen used for inoculation contained a small amount of blood and mucus with active amoebas. They had unsuccessful results, however, from the rectal injections of kittens with freshly passed mucus from a case of typical acute dysentery. Mayer (1919) also inoculated kittens with fresh faecal specimens containing amoebas in the blood and mucus. In the recent experimental work carried on in the Philippines by Sellards and Leiva (1923), dysenteric stools were used to infect a number of kittens. Sellards and Theiler (1924) have recently published their report on a series of experiments in which they infected kittens rectally or by laparotomy with cysts of *E. dysenteriae*. They do not state whether the cysts used were obtained from acute cases or from chronic carrier cases, and they have assumed that an exposure of the stool in the ice-box for thirty-six hours to one week killed the motile phases in the stool.

During the past year, we have attempted to infect kittens by feeding cysts and by rectal injection of faeces containing motile amoebas. We are indebted to Professor Charles A. Kofoid for the specimens, which had been sent to his laboratory for diagnosis and research, as well as for many kindly suggestions. Acknowledgment is also made to Miss Inez Smith, technician for Professor Kofoid.

EXPERIMENTAL AMOEBIASIS IN CATS

MATERIALS AND METHODS

In the vicinity of Berkeley, California, acute dysenteric cases are very rare. The fifteen faecal specimens used for rectal injections were with *one exception* obtained from chronic carriers. To obtain motile forms, it was necessary to give the patient a purge of magnesium sulphate or milk of magnesia. The specimens thus obtained were brought to the laboratory in a thermos bottle and sufficiently diluted with normal saline to draw them up in a syringe. The diluted faecal material was then injected rectally by means of a small rubber catheter inserted four to seven inches within the large intestine. This was done as soon as possible after the specimen had been passed. After the kittens had been thus treated, examinations were made of the remaining portion of the specimen to make sure that motile forms were still present.

When cysts of *E. dysenteriae* were to be fed to the kittens, faeces heavily infected were mixed with milk. If the kittens had not been fed previously on that day, the infected milk was easily taken. In none of the twenty-one kittens which had been fed cysts, did motile amoebas appear in their stools.

ACUTE INFECTIONS IN NORMAL CATS

Unfortunately our first results were complicated by the fact that we reinjected a number of the kittens several times, and four of them had been fed cysts prior to the injections. Our first positive results were obtained after twelve attempts to infect kittens with stools obtained from eight chronic cases. These stools were semi-solid to liquid in consistency and contained no visible blood or mucus. All contained numerous motile amoebas. Case 2 (see table 1) averaged seven *small* actively motile *Endamoeba dysenteriae* and a few cysts to each high power field of the microscope. This specimen was injected into two kittens within fifteen minutes from the time it was passed. On October 4 a specimen from an acute case of dysentery was received. It was liquid, with many flakes of bloody mucus and numerous motile amoebas containing ingested red blood cells. This was the first and only dysenteric stool with which we worked (see table 1). The patient from whom this specimen was obtained had lived in the Philippines for nine years and had had frequent diarrhoea for several years.

TABLE 1

Results of the Rectal Injection of Kittens with Motile Amoebas Obtained from Acute and Chronic Human Cases

No. of specimen	Date of inoculation	Type of case	Cathartic taken	No of kittens injected	No. of previous injections	No. of previous feedings	Results
1a	7/10/23	chronic	milk of magnesia	2	0	0	negative
1b	7/12/23	chronic	milk of magnesia	2	1	0	negative
2	7/14/23	chronic	milk of magnesia	2	2	0	negative
3a	8/21/23	chronic	salts	7	0	0	negative
3b	8/25/23	chronic	salts	7	1	0	negative
4	8/30/23	chronic	salts	5	2	0	negative
5	8/30/23	chronic	salts	5	3	0	negative
6a	9/17/23	chronic	salts	8	3	1	negative
6b	9/20/23	chronic	salts	5	4	1	negative
7	9/20/23	chronic	salts	2	4	4	negative
8	10/ 4/23	acute	none; dysenteric specimen	3	0	0	positive in 9 days
9a	10/ 5/23	chronic	salts	2	5	1	negative
9b	10/ 6/23	chronic	salts	11	5	1	negative
10	1/ 2/24	chronic	salts	1	0	0	positive in 5 days
11	1/ 5/24	chronic	salts	2	0	0	negative

From this dysenteric stool three newly weaned kittens received 15, 20, and 30 c.c. respectively. Nine days later, after saline enemas, the stools of all three kittens showed flakes of bloody mucus in which were actively motile amoebas. Later they developed a bloody dysentery with stools composed of blood and mucus with little or no faecal material. The kittens were closely watched and freshly passed specimens of bloody mucus were used as transfers to other kittens (Wagener, 1924). The strain was carried through six passages in thirty-three cats. Twenty of these animals, which varied in age from young kittens to adults, had not been used in our previous work. The incubation period in the kittens, fifteen in all, was from one to six days. The length of the incubation period appeared to depend upon the age of the kitten and the volume of the stool containing motile amoebas injected. This incubation period was followed by a period of acute bloody dysentery, during which the kittens frequently passed thirty stools a day. Later they were given ether, their blood was used for precipitin tests (Wagener, 1924), and the tissue fixed for histological study.

CHRONIC INFECTIONS IN NORMAL CATS

Five adult and two half-grown cats were infected with strain no. 8 of amoeba from the acute human case. At the time of the infection of these cats, the strain had experienced four to five passages in kittens. After an incubation period of nine days, two of the adults developed acute infections which terminated in death eighteen days after the first appearance of amoebas in their stools. The colon of these two cats was completely ulcerated from the anus to the ilio-caecal valve. Three of the adults and the two half-grown cats developed sub-acute or chronic infections from which they eventually recovered.

On January 3 of this year, a half-grown cat (no. 64) was infected with a thermos bottle specimen obtained after purging with salts from a chronic human case. The cat received 30 c.c. of this specimen. Five days later, flakes of blood and mucus were found in its faeces. These flakes of mucus, which contained a few amoebas, were injected into a two months old kitten. This kitten developed a chronic amoebiasis from which *it also recovered.*

Unfortunately, cat 64, and two of the other adults with chronic amoebiasis, died from a respiratory infection which killed a number of our animals. The necropsy findings for these three chronic cases were absolutely negative. The colon showed no ulceration. The gut was filled with hard faecal material, a condition never found in acute cases.

The remaining animals with chronic infections, together with kitten no. 65, were under continuous observation. Stool specimens were noted daily in regard to color, consistency, and the presence of bloody mucous, and, as frequently as it was possible, microscopical examinations of fresh, warm stools were made for detection of amoebas. It was soon discovered that the amoebas appeared in the stools of these animals intermittently.

The intermittent appearance of cysts of *E. dysenteriae* in the stools of infected individuals has been emphasized by the work of Carter, Mackinnon, Mathews, and Smith (1917). In Professor Kofoid's laboratory, at least six successive faecal specimens are requested in making diagnoses of cysts of *E. dysenteriae.*

The results of the microscopic examination of the stools of these chronic cat cases are shown in table 2.

TABLE 2

Intermittent Appearance of Amoebas in Stools of Cats with Chronic Amoebiasis

Date	Cat 34 (adult)	Cat 35 (half-grown)	Cat 39 (half-grown)	Cat 65 (two mos. old kitten)
Oct. 24	20 c. c. inoculation			
25		8 c. c. inocculation		
27	7 c. c. inocculation			
30		positive	9½ c. c. inocculation	
31		trace B. M.		
Nov. 3		trace B. M.		
5	8 c. c. inoculation		trace B. M.	
7			positive	
9	negative			
10		(a) neg., (b) pos.		
11		negative	negative	
12		positive	negative	
14	enema, trace B. M. positive			
17	negative			
20	negative	B. M.		
21		positive		
22	negative			
23		positive		
24		positive	negative	
26			negative	
28		B. M.		
Dec. 3	enema, trace B. M. positive		enema, negative	
7			negative, B.	
8		positive	negative, B. M.	
9	trace B. M.	positive		
12			trace B.	
14			negative	
19		positive	½ glycotaurate tablet	
29		positive		
31		positive		
Jan. 3		positive	salts	
4		positive		
5		negative		
7		salts, neg.	salts, neg.	
8		negative	negative	4 c. c. inocculation
9		positive	(a) pos., (b) pos.	trace B.
10		negative	positive	
12		negative	positive	
14		positive	positive	

B.—Blood. M.—Mucus.

TABLE 2 (Continued)

Date	Cat 34 (adult)	Cat 35 (half-grown)	Cat 39 (half-grown)	Cat 65 (two mos. old kitten)
Jan. 15		negative	negative	¼ glycotaurate tablet
16			positive	negative
17		negative		positive, B. M.
18				positive, B. M.
19		negative	positive, B. M.	positive, B. M.
21		negative	positive	negative
24		positive	positive	positive
25			B.	
26			B.	
27		negative	B.	
28		negative	B.	
29		negative	B. M., negative	
30				positive
31			negative	positive
Feb. 1		negative	negative	positive
2				positive
4	negative	negative	negative	negative
5		negative	negative, B.	negative
6	negative	negative	negative	(a) neg., (b) pos.
7				(a) pos., (b) pos.
10		positive, B.	negative	positive, B.
11		negative, B. M.	negative	positive
12			negative	negative
13			negative	
14				negative
15		negative		
17				positive
18	negative, B.	negative	negative	
19	negative	negative	negative	negative
20	negative	negative, B.	negative, B.	
21			negative, B. M.	
22	enema, negative	enema, positive	enema, negative	enema, positive
23		negative		negative
24		negative		
25		negative	neagtive	positive
26		negative		
28	trace B. M., neg.			
March 7		negative, B.	negative	negative
8		negative, B.	negative	negative
10			negative	negative, M.
12			negative	negative, B. M.
13		negative	negative	negative, B. M.
14			negative	
15		negative	negative	negative
16			negative	negative
17			negative	
20				negative
21				negative

There is no indication in any of the animals of a cycle of definite duration between the appearances of the amoebas in the faeces. Were cats normal hosts in which cysts were produced, it is possible that such cysts would have been found more frequently and over a longer period than were the motile forms in our cats. Positive findings were reported only when definitely motile amoebas were seen; degenerate forms which are difficult to recognize either in fresh or stained preparations were not reported as positive findings. No cysts were seen.

In acute amoebiasis in cats, the motile forms of *E. dysenteriae* are very large and numerous. We have frequently counted as many as two hundred to a single high power field of the microscope. In chronic amoebiasis, on the other hand, the amoebas are smaller, and a careful search of a flake of bloody mucus is necessary in order to find one or two active organisms.

On April 15, cat 39 was given ether and a necropsy performed. At this time the animal was full grown and in a healthy condition. A considerable amount of fat was present in the mesenteries. No liver abscesses were found. The colon appeared to be scarred from the anus halfway to the ilio-caecal valve. Upon laying the colon open its entire length, the contents were seen to be of a dark brown mucous nature. The inner surface was covered with a film of mucus, but no visible ulcers were present. Smears made from the mucus taken from various regions of the lower part of the small intestine and from the whole of the large intestine were not found to contain amoebas.

Cat 35 was also autopsied April 15. The colon was normal in appearance and was filled with hard faecal material. No amoebas were found at any region. No liver abscesses were present.

Cat 34 was autopsied July 1. The cat was in healthy condition. Considerable fat was present in the mesenteries. The colon was normal in appearance. No amoebas were present.

Cat 65 was autopsied July 1. The animal was fat and healthy. The colon showed the deposition of connective tissue in the region of the rectum. The contents of the colon were semi-solid. No amoebas were found in the smears of a thin film of mucus. The liver was normal, and no ulcers were found in the colon.

The histological findings on the tissues of these four chronic infections will be reported on at a later date.

It is impossible to determine whether Darling's (1913) "senile" forms were obtained from chronic or acute cases, but Baetjer and

Sellards (1914) found that "chronic cases of long standing, with mild symptoms, often produced an attack in animals which was of comparatively short duration and eventually ended in recovery." This we have found true with case 11. These authors suggest the possibility of a difference in pathogenicity, as measured in terms of dysenteric symptoms, of the strains obtained by acute and chronic cases. Our work would again seem to suggest this in the cats. The failure of motile amoebas from chronic cases to infect kittens in thirteen out of fourteen attempts is suggestive of differences in virulence, in tissue penetration, or in organ acclimatization of the various strains in the cat. There is, however, the possibility that these strains from chronic cases produced infection of so mild a type in the cats that they passed unnoticed.

If the cysts used by Sellards and Theiler (1924) in their recent studies on experimental dysentery in cats were obtained from chronic cases, their results differ markedly from ours. These authors obtained their best results through ligation of the bowel, which affords opportunity for the amoebas to penetrate the tissue; under normal conditions they would more readily be expelled before infection took place. Boeck (1924) has also produced experimental amoebiasis in kittens through the rectal injection of culture amoebas. After injection, the anus was sealed over for two days to allow excystation and infection to take place.

INFECTION OF CATS PREVIOUSLY INJECTED WITH AMOEBAE FROM CHRONIC CASES

Prior to the establishment of strain 8, we attempted to infect ten kittens with motile amoebas from chronic human cases of amoebiasis. Subsequently the ten kittens were inoculated rectally with actively motile amoebas from kittens infected with strain 8. These kittens were of the same size and age as many of the normal cats which were used for transfers of this strain, but were far more refractory to infection than the normals were found to be. One half-grown cat (no. 8) had been fed cysts twenty-three times and had received a rectal injection of 50 c.c. of human faeces containing motile amoebas, without contracting the infection. This animal was examined seventeen times over a period of three months and given two warm saline enemas, without blood, mucus, or amoebas showing in the faeces. It was then injected rectally nine times with from 1 to 7 c.c. of bloody mucus containing numerous motile amoebas from infected kittens before a successful transfer was detected. The other nine received from two to seven injections of motile amoebas from acute amoebiasis in cats before they developed experimental dysentery, while one was entirely refractory to infection. Baetjer and Sellards (1914*a*), as well as Dale and Dobell (1918), have found that in the continuous propagation of experimental dysentery there is always a certain per cent of the animals which are refractory to infection. The continuous propagation of our strain no. 8 was not difficult. It was lost only through the lack of an adequate supply of kittens. The twenty normal animals injected rectally with warm, freshly passed stools composed of bloody mucus in which were numerous amoebas gave one hundred per cent positive infections.

While we consider our data insufficient to establish the difference in susceptibility, the difficulties encountered in the infection of the ten cats previously injected with human faeces containing amoebas, in contrast to the ease with which transfers were made to normal animals, is suggestive. It is possible, as we have previously suggested, that these strains of amoebas obtained from chronic human cases produced in kittens infections of such mild type that they passed unnoticed, yet that they called forth a protective immunity on the part of the kitten. This view seems plausible in the light of the extremely mild infection produced by strain no. 10 in cats 64 and 65.

EVIDENCES OF IMMUNITY

This possibility opens up a field of investigation upon immunity to intestinal protozoans in which, so far as is known, no experimental work has been done. Scalas (1923) has found that extracts prepared from bloody mucus containing amoebas, in cases of acute human amoebiasis, will give marked intradermal reactions in cases of human amoebiasis, while normal individuals will not so react. It would seem to us that the reaction would be more specific for the amoebas themselves had he used extracts from infected kittens rather than from man. It is possible that an infected individual becomes sensitive to the by-products of ulceration rather than to the amoebas present in the intestinal ulcers. By using extracts from ulcers in cats, Scalas would escape this complication. We think this may also apply to our own work (Wagener, 1924) in which the senior author obtained precipitin tests, using the serum of cats, which had had symptoms of amoebiasis for a week or longer with an antigen prepared from scrapings of the ulcers, which frequently contained two hundred amoebas to a single field of the microscope.

This same antigen gave a positive precipitin test with the serum obtained from an acute human case of amoebiasis. The test was controlled by the use of a positive serum from a cat which had been infected for fourteen days, by a normal cat serum, and by serum from an individual who was never known to expel cysts of *E. dysenteriae* or to have any clinical symptoms of infection. We were unable to carry on similar serological tests with the blood of chronic human cases.

EXPERIMENTS ON RATS

Kessel (1923) had previously found that amoeba-free rats could be experimentally infected with *E. dysenteriae* by feeding them cysts from human faeces. We therefore attempted to infect rats by the rectal injection of motile amoebas. Sixteen normal amoeba-free rats were injected rectally with from 1 to 4 c.c. of bloody mucus which contained many amoebas obtained from acute cases in kittens. The injections were made by means of a small rubber catheter which was inserted into the colon about 2½ to 6 inches, after the rats had had a heavy dose of salts which cleared the intestine of solid faecal material. As the rats did not develop any symptoms of dysentery, they were given salts fifteen days after inoculation, and examinations were made for the presence of amoebas. None of the rats was found to be infected with *E. dysenteriae*. A laparotomy was performed on four amoeba-free rats, and motile amoebas were injected directly into the caecum by means of a 20-gauge needle which allowed the passage of the amoeba-containing mucus from the infected cats. These four rats failed to develop infections of *E. dysenteriae*.

EXPERIMENTS ON GUINEA PIGS

In 1917 Chatton reported the infection of an adult guinea pig by feeding it cysts from a case of human amoebiasis. Upon the death of the animal from the amoebic infection, two adult guinea pigs were injected rectally with amoebas obtained from the first pig. Chatton states that the material was injected by means of a catheter inserted 15 centimeters within the colon. We therefore gave seven young guinea pigs rectal injections of bloody mucus rich in amoebas from kittens with acute infections. It was with the greatest difficulty that a small, soft catheter could be forced up the colon, which is always, in normal pigs, filled with balls of hard fecal material even after a dose of magnesium sulphate. The amoebas injected were therefore immediately lost by the defaecation of the animal. Inasmuch as the colon of a half-grown guinea pig measures 30 inches from caecum to anus, we doubt if Chatton actually produced infections of *E. dysenteriae* in the caecum of guinea pigs by rectal inoculation. The infection of the caecum would require the passage of the amoebas up the bowel for the distance of at least 24 inches. Twenty-three young guinea pigs each received from four to seven feedings of mature cysts of *E. dysenteriae* from chronic human cases of amoebiasis without a single infection taking place in the guinea pigs thus fed. Two guinea pigs, which had been injected by laparotomy directly into the caecum with amoebas from infected cats, died from a septicemia.

SUMMARY

1. Attempts have been made to infect kittens with motile amoebas from acute and chronic cases of amoebiasis. One acute dysenteric faecal specimen produced infection in three kittens which was carried through six transfers in thirty-three cats.

2. Fourteen attempts to infect kittens with motile amoebas from chronic cases resulted in only one chronic infection which was transferred only once to a young kitten.

3. Amoebas have been found to appear intermittently in the stools from cats with chronic or sub-acute experimental amoebiasis.

4. Kittens which had been previously injected with motile amoebas from chronic human cases were more difficult to infect than normal animals of the same age and size, thus suggesting a possible immunity.

5. No cysts were found in the faeces of infected cats at any time.

6. It has been found impossible to infect guinea pigs and rats by the rectal injection of actively motile amoebas obtained from acute cases of experimental dysentery in kittens.

7. No infections resulted in guinea pigs which were fed cysts from human faeces.

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BINARY FISSION IN OXYRRHIS MARINA DUJARDIN

BY

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INTRODUCTORY AND HISTORICAL

Early in February, 1922, a large flagellate was found in abundance in a sea-water aquarium at the Oakland Pier station of the San Francisco Bay Marine Piling Committee. This organism has been identified as an unarmored dinoflagellate, *Oxyrrhis marina* Dujardin. This species was first described by Dujardin (1841). A few years later this same flagellate was discovered in a marine aquarium at Frankfort by Fresenius (1865), who described it as *Glyphidium marinum.* Saville-Kent (1880–1882) was the first to decide that *Oxyrrhis marina* Dujardin and *Glyphidium marinum* Fresenius were identical, and in his description he retained the older name given the organism by Dujardin. Bütschli was also one of the first authors to recognize the true relationships of *Oxyrrhis;* in an article (1885) published after his monograph appeared is found the following statement:

> Der Kernbau derselben ist . . . . ein feinnetziger und schliesst sich demnach dem Bau der Cilioflagellaten Kerne an. Ich glaube nun, dass sich auch in den sonstigen Bauverhältnissen dieser Form Beziehungen zu den Cilioflagellaten erkennen lassen, welche *Oxyrrhis* noch bestimmter als eine zwischen den Cryptomonaden und Cilioflagellaten . . . . vermittelnde Form aufzufassen gestatten. Er lässt sich nämlich wohl die Möglichkeit erörtern dass von einer Form wie *Oxyrrhis* eine einfache Cilioflagellatenform, wie z. B. *Hemidinium* St., entstanden sei.

Keysselitz (1908) was the first to describe in detail the nuclear division of this flagellate, while the best description of the general morphology is to be found in the excellent paper of Senn (1911), whose observations I have been able to verify in most respects. In none of these accounts, however, have the relations of the neuromotor apparatus and nucleus been described; the rhizoplasts, extranuclear centrosome, and paradesmose have not been found heretofore in *Oxyrrhis,* nor have the chromosomes been accurately followed and the method of their splitting in mitosis determined. The purpose of this paper, then, is to present the first description of the neuromotor system of a dinoflagellate, to trace the nuclear phenomena in the mitosis of *Oxyrrhis,* and to point out certain resemblances of *Oxyrrhis* to flagellates belonging to other groups.

I am indebted to Professor Charles A. Kofoid for suggesting the desirability of studying this flagellate, for his numerous criticisms and suggestions, and for the verification of several of the figures. I wish, also, to thank Dr. Olive Swezy for making an interpretative sketch of figure 6 (pl. 27), and for her valuable suggestions in regard to technique.

MATERIAL AND TECHNIQUE

The original material was obtained from a sea-water aquarium containing a small test-block of piling infested with *Teredo navalis;* this aquarium was supplied with sea water from San Francisco Bay, and was one of a number at the Oakland Pier station of the San Francisco Bay Marine Piling Committee. In company with a species of *Euplotes, Oxyrrhis marina* was found in abundance in the scum on the surface of the water. Material from this culture was used in inoculating a large laboratory aquarium, containing a block of wood formerly infested with *Teredo,* and several smaller aquaria containing sea water and small amounts of dried hay. After a few days the flagellates became abundant, with dividing forms in all the cultures, and they have been present persistently for the last two years.

It has been found that these flagellates can be grown successfully in several different culture media:

(1) Sea water containing a small amount of dried hay; too much hay causes an unfavorable excess of bacteria and ciliates.

(2) Infusions made by boiling dried hay in ordinary sea water.

(3) Infusions made by boiling hay in Van't Hoff's solution (*a*), or a modification of it (*b*):

(*a*)	5/8 M. sol. NaCl	100.00 cc.
	5/8 M. sol. $CaCl_2$	1.00 cc.
	5/8 M. sol. $MgCl_2$	7.80 cc.
	5/8 M. sol. $MgSO_4$	3.80 cc.
	5/8 M. sol. KCl	2.20 cc.
(*b*)	5/8 M. sol. NaCl	96.00 cc.
	5/8 M. sol. $CaCl_2$	2.00 cc.
	5/8 M. sol. KCl	2.00 cc.

(4) Diatom cultures grown in Allen and Nelson's (1910) culture medium, prepared as follows:

Sea water		1000.00 cc.
Solution A		2.00 cc.
KNO_3	20.2 gm.	
Distilled water	100. cc.	

Solution B		1.00 cc.
$Na_2HPO_4 \cdot 12H_2O$	4.0 gm.	
$CaCl_2 \cdot 6H_2O$	4.0 gm.	
$FeCl_3$ (melted)	2.0 cc.	
HCl (concentrated)	2.0 cc.	
Distilled water	80.0 cc.	

After mixing the sea water with solutions A and B, the mixture is heated to 70° C., cooled, and then the clear supernatant liquid is decanted.

Of the methods listed above, the last is probably the best, since the diatom cultures are much less favorable to the growth of ciliates and at the same time provide abundant food for the flagellates.

In most of my cultures dividing flagellates have been fairly numerous at any hour of the day or night, although they appear to be slightly more abundant in the early evening and early morning. As a rule, the organisms are found in greater numbers in the surface film than in the deeper portions of the culture. In association with *Oxyrrhis* in my cultures, *Euplotes*, *Uronychia*, *Lionotus*, *Loxophyllum*, *Uronema*, *Oxytricha*, and a small *Amoeba* have occurred frequently. In the older cultures especially, both vegetative and encysted stages of amoebas are found in great numbers.

All preparations have been made by the cover-glass method. In most cases, covers smeared with Mayer's albumen-fixative have been floated on the surface of the cultures; on removal, the covers are placed with the adherent organisms uppermost and the fixing fluid pipetted onto them. In other cases, material has been killed in mass after centrifuging, and then run up on cover slips in the usual way. Various killing fluids have been tried, including Bouin's, Zenker's, Schaudinn's (both hot and cold solutions), strong Flemming's, weak Flemming's, and a mixture of Flemming's fluid with from 10 to 15 parts of a modified Van't Hoff's solution (explained above). The most satisfactory of these have been the Flemming's mixture, Flemming's strong, and hot Schaudinn's fluid, with the first probably the best of the three. In staining, Delafield's haematoxylin, aqueous and alcoholic iron-alum haematoxylins, Mallory's triple stain, Mann's mixture, and various counterstains—light green, acid fuchsin, Bordeaux red, Congo red, neutral red, orange G, methyl green and eosin—after the haematoxylins have been used. The iron-alum haematoxylins have given the best results with the nucleus, while Bordeaux red and Congo red have proved to be the best stains for flagella and rhizoplasts.

GENERAL MORPHOLOGY

Shape and Size

The body of *Oxyrrhis* is an elongated ovoid structure (figs. A, B), widest at the girdle and slightly flattened dorsoventrally. The *epicone* is ovoid, with the *apex* broadly rounded, while the *hypocone,* containing a deep ventral excavation (the *sulcus*), is deficient on the left side with the antapex displaced to the right and bluntly rounded. In relative dimensions the length is usually from 1.5 to 2.0 times the greater diameter, rarely 2.5 transdiameters. At its shortest point, just to the left of the *tentacular lobe*, the epicone extends for about 0.5 the total length, while on the right flank, where the girdle fades out, its length is about 0.8 that of the body.

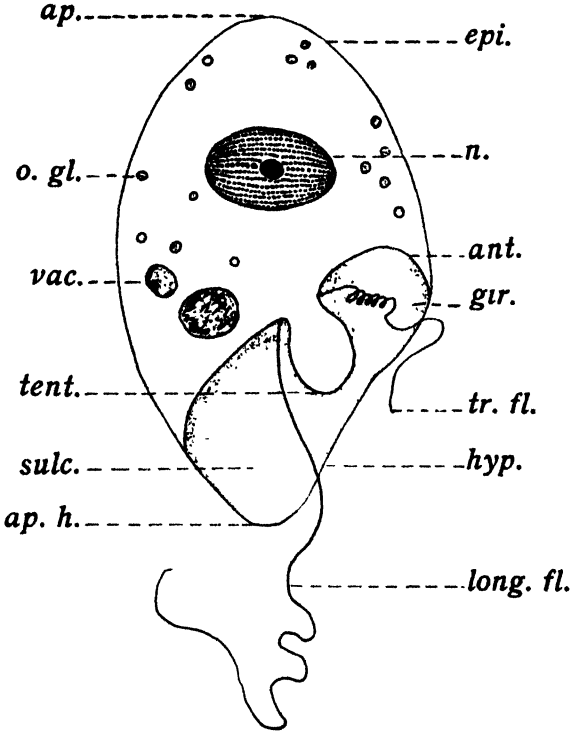

Fig. A. Diagrammatic camera lucida sketch from living *Oxyrrhis marina*, ventral view; *ant.*, anterior margin of girdle; *ap.*, apex; *ap. h.*, antapex; *epi.*, epicone; *gir.*, girdle; *hyp.*, hypocone; *long. fl.*, longitudinal flagellum; *n.*, nucleus; *o. gl.*, oil globules; *sulc.*, sulcus; *tent.*, tentacular lobe; *tr. fl.*, transverse flagellum; *vac.*, food mass in vacuole. × 1750.

The *girdle* is incomplete, extending from the tentacular lobe obliquely to the left and posteriorly, thence on transversely around the dorsal side to the right margin of the body, where it fades out after having made about 0.7 of a revolution. The *anterior lip* of the girdle is sharply defined but the posterior lip is lacking. The *sulcus* is present on the ventral side of the hypocone as a broad excavation lying to the right of the tentacular lobe and continuous anteriorly at the left with the girdle.

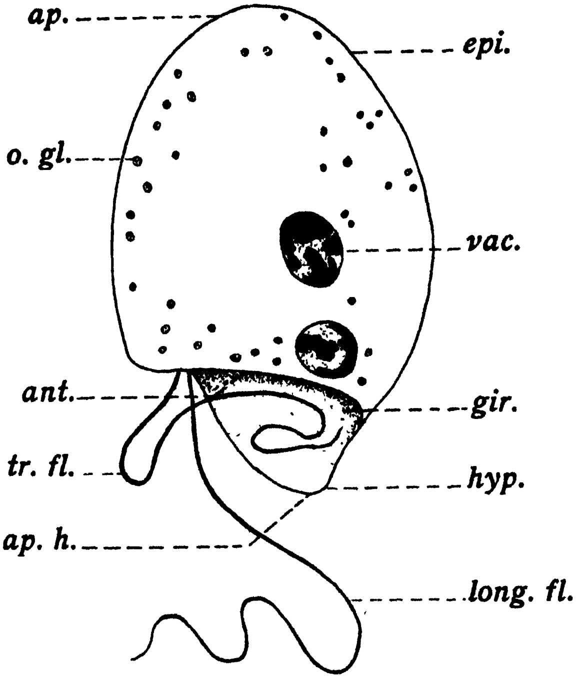

Fig. B. *Oxyrrhis marina*, diagrammatic camera lucida sketch from living; dorsal view; labeling as in figure A. × 1750.

The tentacular lobe, which is homologous with the more specialized *tentacles* of other dinoflagellates (Kofoid and Swezy, 1921), is a pear-shaped, lobate structure lying between the two flagellar pores, and attached by its base to the anterior lip of the girdle, and possibly to the ventral side of the hypocone. This organelle is said to be contractile (Kofoid and Swezy, 1921); this statement is supported by the extreme variations in length (fig. C) frequently found in my material, but actual contraction or extension of the lobe has not been observed in the living flagellates. These differences in length may therefore be due to growth phenomena, rather than to contraction or extension of that organelle. This structure was first recognized as a lobe by Blochmann (1884); previous investigators (Dujardin, 1841; Fresenius, 1865; Cohn, 1866; Kent, 1880–1882) had interpreted it as a vacuole, or other internal structure.

Oxyrrhis varies greatly in size, extremes of 10μ (Keysselitz, 1908) and 50μ (Dujardin, 1841) having been recorded. In our cultures the average range in length is from 25 to 35μ, with extremes of 12 and 45μ. The width varies from 8 to 26μ.

The asymmetrical shape of the body of *Oxyrrhis* doubtless accounts for many of the variations in the figures presented in the descriptions of the various authors. Gourret and Roeser (1886) were even led to the erroneous conclusion that the body is metabolic. Senn (1911) has worked out very carefully, with the aid of wax models, the morphology of *Oxyrrhis*, and his paper includes the best existing description of the shape and general structure of the body; in general, my own observations support his description.

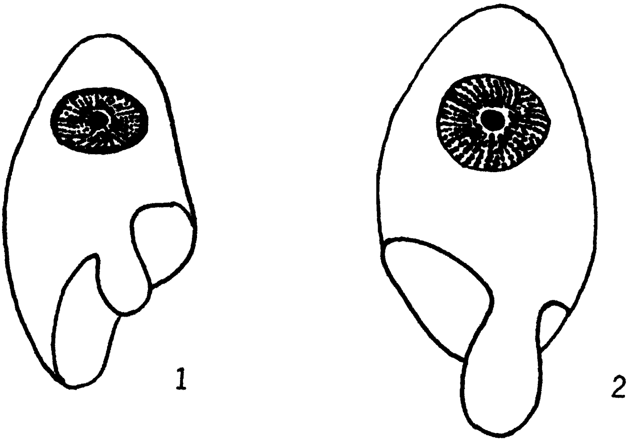

Fig. C. *Oxyrrhis marina*, diagrammatic camera lucida sketches showing variations in relative length of the tentacular lobe; (1) ventro-lateral view; (2) ventral view. × 1770.

The body of *Oxyrrhis* is enclosed in a definite ectoplasmic wall, or periplast, which frequently appears double-contoured and in the larger forms seems to be about 0.5μ thick. In the living flagellates the endoplasm is seen to contain numerous fine granules and larger inclusions. After fixation and staining, a finely alveolar structure frequently appears in combination with the granular endoplasm. Suspended in the endoplasm of the living organisms are numerous small oil globules (figs. A, B; pl. 26, figs. 1, 2), which are usually more abundant toward the periphery of the cell. Also food masses of various sizes are present, as a rule, enclosed in food vacuoles (fig. D; pl. 26, figs. 1, 2, 4, 5); these occur regularly in the posterior part of the body, but are more or less common in the anterior part. The vacuoles formed around ingested diatoms and algae are frequently large, not uncommonly extending from one end of the animal to the other, as Senn (1911) has also mentioned. Senn has noted, in addition, a fluid vacuole, perhaps comparable with the *pusule* of many dinoflagellates, but no canals extending to the flagellar pores could be made out. I have often observed vacuoles containing no solid food masses, but it does not seem certain that they should be homologized with the pusule of other forms. Despite the assertion of Cohn (1866) and Kent (1880–1882), a contractile vacuole is apparently absent in *Oxyrrhis;* continued observation has failed repeatedly to reveal such a structure, and several other investigators (Blochmann, 1884; Senn, 1911) have also been unable to find one.

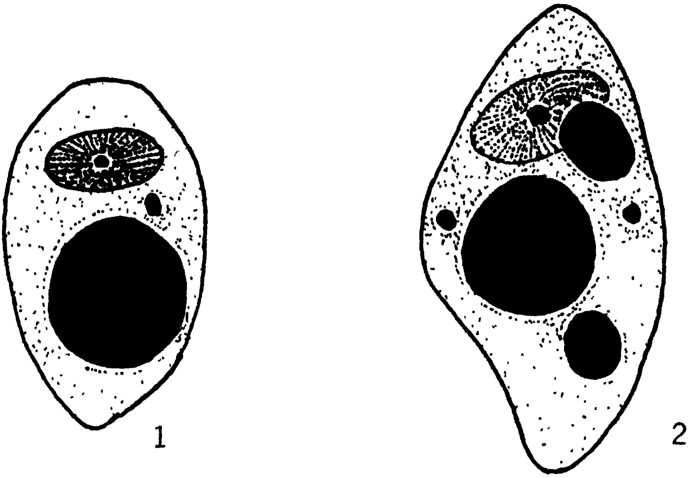

Fig. D. *Oxyrrhis marina,* diagrammatic camera lucida sketch showing food masses in vacuoles; flagella not shown; stained with iron-alum haematoxylin. × 1770.

Dunkerley (1921), in one of his figures (fig. 7) of *Oxyrrhis,* shows what he terms "parasitic spores" in this flagellate; these structures are located in the posterior half of the body, apparently in vacuoles, and resemble somewhat the usual masses of partially digested food enclosed in food vacuoles. In his paper, Dunkerley figures none of the food vacuoles commonly observed in these flagellates, and it is obvious that, with a holozoic organism such as *Oxyrrhis,* any indications of parasitism should be thoroughly investigated before a conclusion is reached, since a misinterpretation of ingested food material is quite possible.

NEUROMOTOR SYSTEM

The neuromotor system (fig. F) of *Oxyrrhis* consists of two *flagella* which end in *blepharoplasts* just beneath the ectoplasm at the base of the tentacular lobe, two *rhizoplasts* extending from the blepharoplasts to an *extranuclear centrosome*, and there is some evidence that an *intranuclear rhizoplast* (pl. 27, figs. 7, 9; pl. 28, fig. 12) connects the centrosome with the endosome. It has been possible to detect only one rhizoplast, even in the early metaphase stages in which this structure has been observed; whether the rhizoplast divides or not could not be determined. At the beginning of binary fission, a *paradesmose* appears as a fine thread drawn out between the two daughter centrosomes after their division.

Oxyrrhis possesses two flagella (figs. A, B; pl. 26, figs. 1, 2), a longer *longitudinal flagellum* inserted on the right side of the tentacular lobe, and a shorter *transverse flagellum* inserted on the left side. The longitudinal flagellum is from 1.5 to 2.0 times as long as the body, while the transverse flagellum is only a little longer than the body. Each flagellum has been found to end in a *blepharoplast* near the base of the *tentacular lobe,* as described by Keysselitz (1908) and Senn (1911); in addition, a rhizoplast extends from each blepharoplast to the *extranuclear centrosome* just outside the nuclear membrane (fig. F).

As Kofoid and Swezy (1921) have stated, there exists in the dinoflagellates not only a functional but usually also a structural differentiation between the two flagella, in that the transverse flagellum is frequently, if not always, ribbon-like. In a primitive form like *Oxyrrhis* one might hope to find the beginnings of such a structural specialization. Keysselitz (1908) has found that the flagella are somewhat similar; both appear flattened, gradually diminishing to a thread toward the point, and consist of a *cytoplasmic sheath*, which does not extend quite to the tip of the flagellum, enclosing a uniformly heavy *axial filament* which lies toward one side of the sheath. Such a structure is not unlike that of the typical transverse flagellum of more highly specialized dinoflagellates. in which "the transverse flagellum itself consists of a stout fibril, bordered on one side by a comparatively wide, fin-like sheet of transparent protoplasm or membrane'' (Kofoid and Swezy, 1921). Senn (1911) also observed the axial filament, but was unable to determine whether it is axial or lateral in position; he found that both flagella are similar in structure.

Although it has been impossible in my material to make out with certainty any cytological difference in the structure of the two flagella, in flagellates killed in formalin the transverse flagellum is found to be typically coiled while the longitudinal flagellum remains more or less extended. Kofoid has informed me that such a condition is characteristic for all the other dinoflagellates which he has observed. It seems obvious that such a difference in fixation must depend upon some fundamental difference in structure. Therefore it is to be concluded that this diagnostic feature of the dinoflagellates has already taken place, even though slight, in *Oxyrrhis*—namely, a structural differentiation of the two flagella into the longitudinal and the transverse. This differentiation is distinctly evident with regard to the function and behavior of the two flagella, since each is strikingly similar to the corresponding flagellum of more highly differentiated dinoflagellates; the transverse flagellum lies in the girdle, while the longitudinal flagellum extends posteriorly along the sulcus and is trailed behind the flagellate.

Keysselitz (1908) states that the flagella may be cast off at the slightest injury to the animal and that they often carry their blepharoplasts with them; I have not been able to confirm the loss of the blepharoplasts in such cases, but I agree with Senn (1911) that those flagellates which do cast off their flagella soon disintegrate; hence, such behavior is probably not autotomy, but rather a step in the changes preceding death of the organism.

THE NUCLEUS

The nucleus of *Oxyrrhis* is an ovoid structure lying usually somewhat anterior to the middle of the body (pl. 26, figs. 3–5); it varies in size with that of the organism, the average length of the longer axis ranging from 7 to 10μ. A distinctly staining nuclear membrane is present both in the resting nucleus and throughout mitosis.

Near the center of the nucleus lies the *endosome* (pl. 26, figs. 3–5), which is spherical in the resting and early prophase stages. This body is the ''Nucleolo-Centrosoma'' described by Schaudinn (1896), and by him homologized with the endosome of *Euglena*. In addition to resembling the endosome of euglenoids, the endosome of *Oxyrrhis* is quite similar to the "Caryosom" of *Gymnodinium* (Jollos, 1910), and possibly comparable with the sphere of *Noctiluca* (Calkins, 1899; Doflein, 1900).

Around the endosome the chromatin appears in the form of chromomeres, arranged in string-of-beads fashion in rows, or chromosomes (pl. 26, figs. 3–5); such an organization seems to be evident even in the resting nucleus, as characteristic of the dinoflagellates "where there appears in the nuclei . . . . to be a persistent organization of beaded chromosomes with subparallel or even spiral arrangements within the nucleus" (Kofoid, 1923).

HABITS AND DISTRIBUTION

Distribution

Oxyrrhis marina is widely distributed, having been previously reported from the coasts of Europe, South America, and eastern North America. In addition to the occurrence in San Francisco Bay, *Oxyrrhis* was found by the writer in a small tide pool, and also by Dr. C. V. Taylor in a standing sea-water culture at Hopkins Marine Station, Pacific Grove, California, during the summer of 1923.

Orientation and Locomotion

Before the true affinities of *Oxyrrhis* were known, this organism was classified with *Chilomonas* in the Cryptomonadina; hence, in homologizing this form with its supposed relatives, it was necessary to designate the flagellar end as the anterior one. With the realization of the dinoflagellate relationships of *Oxyrrhis* this necessity no longer prevailed; hence the anterior end is now considered (Senn, 1911; Kofoid and Swezy, 1921) to be the end which retains that position in locomotion, and the longitudinal pulsellar flagellum is thus directed posteriorly, as in other dinoflagellates.

Owing to the spiral rotation and the rapidity of locomotion of this flagellate, the accounts of the earlier investigators vary somewhat with regard to the functions of the two flagella in locomotion. As described by Kent and Senn, one flagellum (the longitudinal flagellum, figs. A and B) is stretched out posteriorly in locomotion, effecting by means of its spiral undulations forward movement of the organism. The second flagellum (transverse flagellum), through its rapid lashing, causes the rotation of the body which is so characteristic of a swimming *Oxyrrhis*. This rotation, combined with the asymmetrical structure of the body, results in locomotion in a spiral path, a method common in flagellates and typical of the dinoflagellates, as pointed out by Kofoid (1910):

> .... It is on the left side of the body that the flagella originate or to which the transverse one always turns, and it is always this side that is the longer or exhibits the greater structural development. Fundamental asymmetry thus attends the arrangement of the flagella. . . . Throughout the group the transverse girdle is usually .... wound about the body in a descending left spiral. . . . Types of asymmetry such as these .... are plainly correlated with this spiral course in locomotion ....

Senn (1911) also suggests that the transverse groove (the *girdle*, figs. A, B; pl. 26, figs. 1, 2) which extends posteriorly and to the left not only facilitates rotation but also determines the pitch of the spiral path; while the longitudinal flagellum, in addition to driving the organism forward, determines the longitudinal axis of rotation. Both Keysselitz and Senn have mentioned the fact that these flagellates often cease rotating during locomotion—in such cases the longitudinal flagellum alone is active, while the transverse flagellum remains coiled up in the girdle as when the organism is at rest—and Senn (1911), on the basis of two observed instances of ingestion of food particles, has suggested that this type of locomotion is correlated with feeding activities. Occasionally, there also occurs a reversal in the direction of locomotion, as Kent (1880–1882) and Senn (1911) have already noted; this reversal is thought by Senn to be occasioned by some undetermined change in the activities of the transverse flagellum.

NUTRITION

Oxyrrhis is holozoic and is rather omnivorous in feeding habits, having been reported to ingest small flagellates as well as bacteria, algae, and diatoms. Keysselitz (1908) found masses of the alga *Spirogyra* coiled up in the food vacuoles, and other investigators (Fresenius, 1865; Senn, 1911) have observed ingested bacteria and diatoms. A striking feature is the ability of these flagellates to ingest large food bodies; Senn (1911) has recorded the ingestion of a *Navicula* which reached from one end of the body to the other.

Similar examples have been noticed in my diatom cultures, where the flagellates are frequently stretched into grotesque shapes by the unyielding food masses which they contain. After digestion has proceeded for some time, these materials are gradually broken down into spherical masses which tend to accumulate as a rule in the posterior part of the body (fig. D; pl. 26, figs. 4, 5). In many of the individuals observed, there is one large vacuole posterior to the nucleus, varying in size but often extending from the dorsal wall on down to the ventral wall of the cell; in addition, there may be several smaller vacuoles.

Kent (1880–1882) has reported ingestion of food as taking place when the organism is at rest, the lashing of the transverse flagellum serving to drive the food particles toward the mouth. This author was also able to observe the ingestion of carmine particles, but no other investigators have obtained positive results in similar experiments. Senn (1911) was unable to verify Kent's description of food-taking; in fact, in the only case of ingestion observed by him, the flagellates were swimming about without rotating, circling about the food with the ventral side of the body turned toward it.

According to Blochmann (1884), after the completion of digestion the waste materials are expelled from the tip of the antapex:

> . . . . Dieser Körper ist nun ein Excretballen, wie sich leicht zeigen lässt. Setzt man nämlich ein Thierchen, das einen grossen Excretballen enthält, nur einem ganz leisen Druck unter dem Deckglasse aus, so bemerkt man oft, dass dieser Ballen sofort ungefähr an der Spitze des Fortsätzes ausgestossen wird. Es bleibt dann längere Zeit noch eine Einsenkung zurück, die sich allmählich verschliesst.

Although this method of excretion seems probable enough, no such process has been determined in my material. In a few cases (pl. 29, figs. 16, 17), a vacuole, either empty or containing heavily stained material, has been seen near the tip of the hypocone in fixed material, but whether this structure has any connection with 'excretion' could not be determined.

A few investigators have reported a contractile vacuole in the posterior end of the body, but the presence of such a structure is denied by Blochmann (1884) and Senn (1911). In my material, also, it has been impossible to determine a contractile function for any of the numerous vacuoles in *Oxyrrhis.*

ENCYSTMENT

Encystment of *Oxyrrhis* has not been described heretofore, but from my cultures there have frequently been observed flagellates which have lost the flagella and developed a definite 'cyst wall' (fig. E). In every case these flagellates have contained either a large food vacuole or several smaller ones filled with partially digested food. Such a type of encystment belongs to the second type described by Kofoid and Swezy (1921) as characteristic of marine dinoflagellates:

> . . . . Encystment in the dinoflagellates, at least in the marine forms, is probably due to one of two causes. The first of these is the need for protection during the non-motile stage of the period of multiple and binary fission. Binary fission may occasionally take place in non-encysted forms, but it is far more frequently seen in encysted ones. . . . The second cause of encystment is apparently induced by the ingestion of large food bodies, and indicates a need for a quiescent period for its assimilation.

Only the second type of encystment has been observed in *Oxyrrhis;* multiple fission is not known in this organism, and binary fission appears to occur only in actively motile stages. The method of encystment in *Oxyrrhis,* as observed in my material, is in accord with the general description of cyst formation in dinoflagellates (Kofoid and Swezy, 1921):

> The cyst is first formed as a thin membrane secreted by the body wall and closely investing it. The flagella are apparently lost at the beginning of the process and the membrane is closely adherent to the outlines of both girdle and sulcus. The flagella are probably absorbed. . . . Very soon after the formation of the membrane the cyst begins to enlarge as a result of the incoming fluids which fill the spaces between the cyst wall and the body.

A series of test cultures carried out with *Oxyrrhis* indicates that encystment may possibly occur as a result of unfavorable conditions. A number of hanging drop cultures in depression slides sealed with vaseline were started in moist chambers. During the first two weeks division progressed at a fairly normal rate, but with a remarkable decrease in size, so that at the end of about three weeks the flagellates were only about one-third as large as the original ones placed in the cultures. At the end of this period a few non-motile forms were observed in each culture, all with the characteristic appearance of the feeding cysts except that the food bodies were either very small, or else lacking altogether. After six weeks, exclusively non-motile forms were observed; in these, no flagella could be detected in observation under the oil immersion objective, and in some cases a 'cyst wall' seemed to surround the flagellates. Since there was no appreciable evaporation of the hanging drop, the possibility of plasmolysis due to increased salinity of the water seems to be removed.

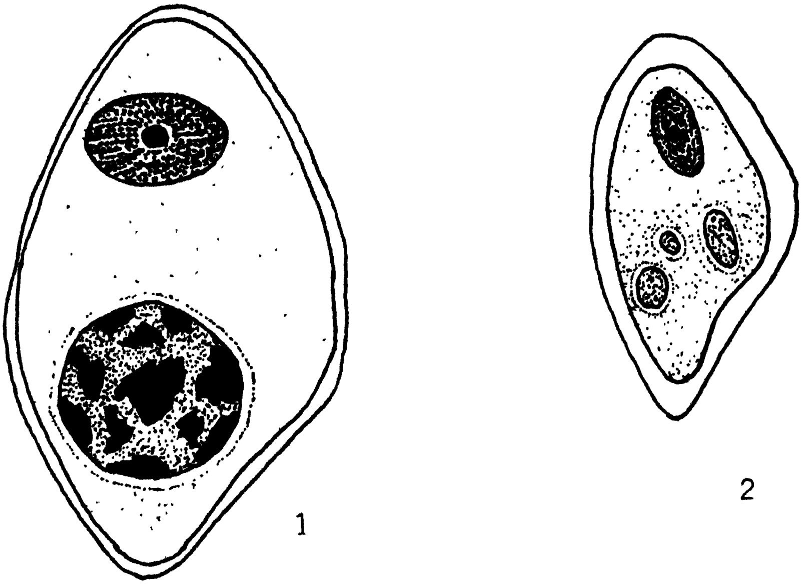

Fig. E. *Oxyrrhis marina*, camera lucida sketches of 'cysts'; note absence of flagella. Iron-alum haematoxylin. × 1770.

BINARY FISSION

NEUROMOTOR APPARATUS

Binary fission in *Oxyrrhis*, as in other flagellates, is initiated by the division of the extranuclear centrosome (fig. F, 2; pl. 26, fig. 3). As the two daughter centrosomes draw apart along the nuclear membrane, they remain connected by a fine fibril, the *centrosome-paradesmose*. Occasionally, also, one of the extranuclear centrosomes is joined to the endosome by another fibril, the *intranuclear rhizoplast* (fig. F, 1–4; pl. 27, figs. 7, 9; pl. 28, fig. 12). A splitting of this intranuclear rhizoplast in mitosis could not be determined.

In the late prophase (fig. F; pl. 27, fig. 7), after the daughter centrosomes have become further separated, two new flagella appear. So far, the behavior of the neuromotor apparatus in *Oxyrrhis* does not differ from the behavior of that in the Euflagellata during mitosis; that is, the early steps seem to be in preparation for an ordinary longitudinal fission. In the metaphase (fig. F, 4; pl. 27, fig. 8), however, there occurs a shifting of the nucleus and neuromotor apparatus, so that the paradesmose now comes to lie parallel to the major axis of the body instead of at right angles to it, as in the prophase (fig. F, 2, 3). During the metaphase the centrosomes migrate to their final position at the poles of the nucleus, drawing the paradesmose out between them (fig. F, 4, 5; pl. 27, figs. 9, 10; pl. 28, figs. 11–14). The paradesmose persists throughout the anaphase (fig. F, pl. 29, figs. 17, 18), but is broken at the beginning of the telophase (fig. F, 7). In the late telophase (fig. F, 8; pl. 30, figs. 22–24) the neuromotor apparatus of the anterior schizont becomes shifted posteriorly from its earlier position (fig. F, 7; pl. 30, fig. 20) to approximately the position it is to occupy in the daughter organism.

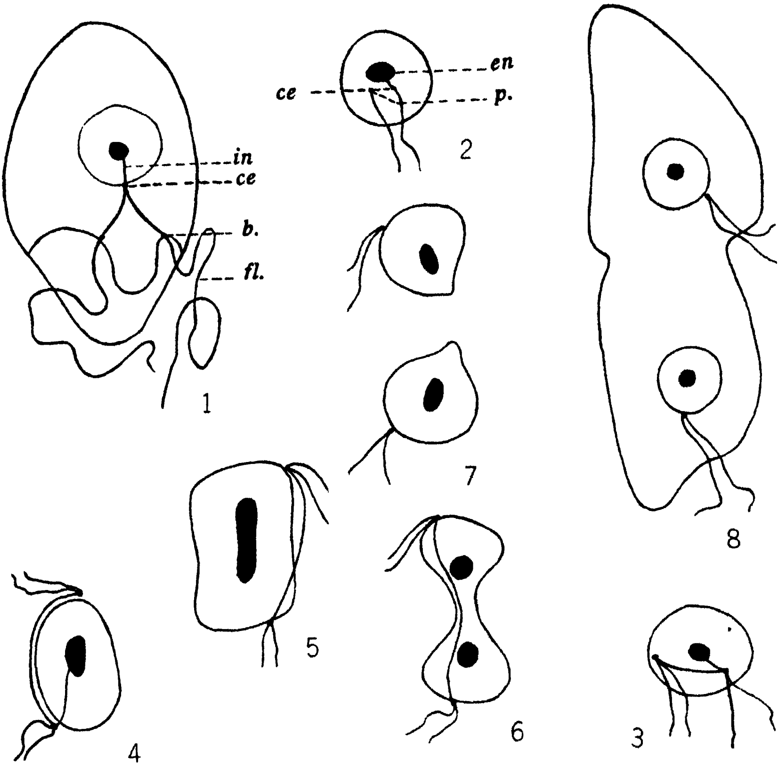

Fig. F. *Oxyrrhis marina*, diagrammatic camera lucida sketches showing the behavior of the neuromotor apparatus during binary fission; the chromosomes are not indicated; figures 2–7 show only the nuclei, in their relative positions. Prophase (2, 3), metaphase (4), anaphase (5, 6) and telophase (7, 8) stages are shown. *b.*, blepharoplast; *ce.*, extranuclear centrosome; *en.*, endosome; *fl.*, flagellum; *in.*, intranuclear rhizoplast; *p.*, paradesmose. × 1150.

RESTING STAGE

The designation of different stages in the mitosis of *Oxyrrhis* must necessarily be somewhat arbitrary. For convenience in discussion, the resting stage (or interphase) is considered to extend from the completion of the telophase until the division of the extranuclear centrosome; the prophase to end with the appearance of two new flagella before the shifting of the nucleus; the metaphase, to extend from the rotation of the nucleus to the separation of the daughter chromosomes in the equatorial plane; the anaphase, to the separation of the daughter nuclei; and the telophase, to the completion of binary fission.

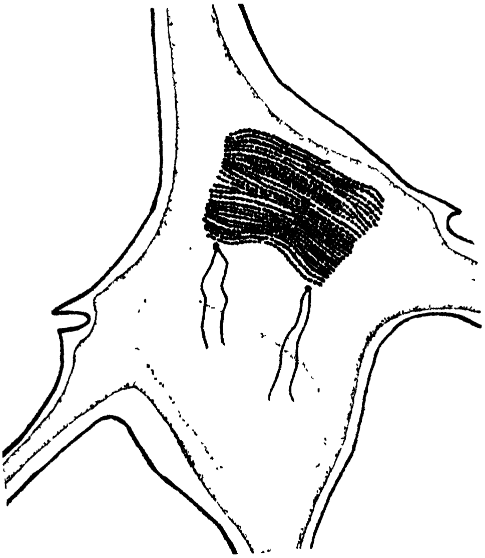

Fig. G. *Ceratium hirundinella*, diagrammatic camera lucida sketch, showing flagellar rhizoplasts ending in granules just outside the nuclear membrane; nucleus in metaphase stage. × 890.

The nucleus of the resting stage and prophase is an ovoid structure lying in the middle or anterior part of the body, and enclosed in a distinct and persistent nuclear membrane (pl. 26, fig. 3). It contains a more or less centrally located *endosome* which, with regard to its behavior in mitosis, is comparable with a similar structure in *Gymnodinium fucorum* (Jollos, 1910), with the endosome of euglenoids (Hall, 1923), and perhaps also with the sphere of *Noctiluca* (Calkins, 1899; Doflein, 1900). Jollos (1910) in two of his figures (pl. 9, figs. 49, 50), shows a somewhat similar body in the dividing nucleus of *Ceratium tripos*, although here there are also several other karyosomes which do not seem to divide in mitosis.

Keysselitz (1908) has figured a centriole in the endosome of the resting nucleus in *Oxyrrhis*, and Jollos (1910) also describes a centriole in the endosome of *Gymnodinium fucorum.* The presence of a centriole in *Oxyrrhis* is denied by Senn (1911) and Dunkerley (1921), and could not be confirmed in my material. In some cases, the intranuclear rhizoplast has produced in optical cross-section the appearance of a 'centriole'; such a structure has been seen a number of times, and may possibly account for the description published by Keysselitz.

The chromatin material is distributed around the endosome in the form of chromomeres composing beaded chromosomes (pl. 26, figs. 3–5). So far as can be determined, this structural feature of the chromatin is a constant condition in the nucleus of *Oxyrrhis.* A striking feature of the nucleus of *Oxyrrhis,* and of some other dinoflagellates as well, is the apparent absence of a 'resting nucleus,' in the sense conveyed by the term as used in Metazoan cytology. Instead of a fading out, or a decrease in the amount of chromatin as the telophase proceeds to the interphase there is a persistence of the telophase organization of the beaded chromosomes, although they perhaps lose something of their parallel arrangement. In the resting nucleus there seems to be no definite arrangement of the coiled chromosomes, whereas in the prophase (pl. 26, figs. 4. 5) a distinctly radial arrangement is evident. During this stage, and to the end of the prophase, the long axis of the nucleus lies in a transverse plane through the body. The resting nucleus of Senn (1911) appears to correspond to an early metaphase or late prophase, since the position of the nucleus is shifted, and the length is increased.

THE PROPHASE

The prophase is initiated by the division of the extranuclear centrosome (pl. 26, fig. 3). Keysselitz (1908) states that mitosis is begun by the division of a centriole contained in the endosome; as mentioned above, the presence of a centriole in the endosome of *Oxyrrhis* has not been confirmed, and there is a possibility that an intranuclear rhizoplast has been misinterpreted by Keysselitz as a granule in the endosome.

It is difficult to understand Dunkerley's (1921) statement that "*the first appearance of division is shown by the appearance of two karyosomes, the actual method of their formation not being seen.*" In my material, two separate endosomes have not been observed in the nucleus until the late anaphase. In a stage corresponding to what we have termed the anaphase, Dunkerley goes on to say: "*Most important is the behavior of the karyosomes at this stage. They appear to be connected by a distinct central strand, in spite of the fact that two separate karyosomes are seen in earlier stages.*" And later, in the telophase, "*there are now two karyosomes present, presumably formed by the snapping across of the central axis.*" A fragmentation of the endosome is not impossible in *Oxyrrhis;* such a phenomenon has been described in euglenoids, and Dunkerley's case of two endosomes in the early stage of mitosis in *Oxyrrhis* might be merely another example of fragmentation of the endosome. Dunkerley's figure 3 clearly indicates, as do similar figures of Keysselitz (1908), that the division of the endosome is not completed until the end of the anaphase, just as it has been observed in my material.

Senn (1911) considers that division is anticipated by a shifting of the position of the nucleus so that it lies transversely in the body; from my observations, however, it seems that the shifting in the prophase is just the reverse, and that Senn's supposed resting nucleus represents in reality a very late prophase stage.

In the early prophase (pl. 26, fig. 3) the chromosomes are somewhat loosely coiled around the endosome, and the nucleus stains less densely than in the later stages. Toward the end of the prophase, there is observed the beginning of a definite arrangement of the chromosomes into more or less parallel rows. The frequent appearance of a radial arrangement of the chromosomes (pl. 26, figs. 4, 5) with the ends converging toward the centrosomes suggests that in the prophase there is a polarization of the nucleus. It is possible that this may be correlated with the existence of an intranuclear rhizoplast, as Boeck (1917) has shown to be the case in *Giardia.* After the separation of the daughter centrosomes, elongation of the nucleus begins, the nucleus still lying in a transverse plane of the body.

THE METAPHASE

In the late prophase or early metaphase, splitting of the chromosomes begins; as is the rule in flagellates, the splitting is longitudinal. During this phase of mitosis the nucleus shifts to its final position, lying parallel to the major axis of the body. The splitting seems to begin at one end of each prophase chromosome; as the split lengthens, the freed ends of the daughter chromosomes separate and begin to migrate to opposite poles of the nucleus (pl. 27. figs. 8, 9; pl. 28, figs. 11–13), resulting finally in the production of an 'equatorial plate' (pl. 27, fig. 10; pl. 28, fig. 14) similar to that found in *Ceratium* and *Noctiluca* (Calkins, 1899; Doflein, 1900). It should be emphasized that in this 'equatorial plate' (pl. 27, fig. 10; pl. 28, fig. 14) the chromosomes, as rows of chromomeres, lie parallel to the long axis of the nucleus. This essential characteristic of the metaphase nucleus of *Oxyrrhis* is not indicated at all in the figures of Keysselitz (1908); in fact, the nuclei in his figures seem to contain chromomeres only, and no distinct chromosomes. Dunkerley's (1921) figures suggest the parallel arrangement of the chromosomes in the metaphase and anaphase, but he seems to have overlooked entirely the beaded structure so characteristic of the chromosomes in *Oxyrrhis* and other dinoflagellates.

In one case (pl. 28, fig. 13), a noticeable difference in the two poles of the nucleus is seen; at the anterior end the chromosomes have not yet converged to a point, but seem to extend to a clear area of cytoplasm. This condition is quite similar to that at the ends of the amphiaster of *Noctiluca* (Calkins, 1899, pl. 42, fig. 31), the clear area of cytoplasm resembling a 'centrosphere' of metazoan cells (Chambers, 1917). Another feature which may be correlated with the observations of Chambers (1917) on the sol-gel phenomena in mitosis is the frequent occurrence of a seemingly non-granular or less densely stained area around the nucleus (pl. 26, fig. 3; pl. 27, figs. 8, 9; pl. 28, figs. 11, 12, 14; pl. 30, figs. 22–24), indicating a difference in the physical structure of the cytoplasm in this region. This hyaloplasmic area around the nucleus is also figured by Dunkerley (1921, figs. 2–6), and in two figures by Keysselitz (1908, pl. 19, figs. 10, 11).

In the late metaphase the endosome becomes elongated (pl. 27, fig. 10; pl. 28, fig. 14), as in euglenoids (Baker, 1921; Hall, 1923) and in *Gymnodinium* (Jollos, 1910). In one case (pl. 28, fig. 14) abnormal constriction of the endosome, probably caused by the marked constriction of the nucleus, was observed.

Counts of the parallel chromosomes in the equatorial plate have given 17, 18, 19, and 20 in different cases, indicating a total number of about forty chromosomes; this is in accord with counts of optical cross-sections of nuclei in the prophase, in which from thirty-five to forty-two have been determined.

THE ANAPHASE

With the beginning of the anaphase the two daughter centrosomes, each with its pair of flagella, are approaching the poles of the nucleus (pl. 29, figs. 15, 16); the paradesmose is still present (pl. 29, figs. 17, 18). The daughter chromosomes have separated in the equatorial plane, and the two daughter plates have begun to move apart as elongation of the nucleus proceeds (pl. 29, fig. 16). The endosome also becomes more elongated, with the result that it is drawn out between the two groups of chromosomes, resembling the endosome of euglenoids at a corresponding stage of mitosis (pl. 29, fig. 17). Dunkerley (1921, fig. 2) figures what appears to be an early anaphase (resembling somewhat my figure 15, on pl. 29), in which there are two separate endosomes, one in each daughter belt of chromosomes. In a corresponding stage (pl. 29, fig. 15) I find that the endosome is not divided, but extends from one group of chromosomes to the other. Dunkerley's later anaphase (fig. 3) corresponds closely to the stages I have observed (pl. 29, figs. 16, 17).

At first parallel to the endosome in the early anaphase (pl. 29, fig. 16), the separated ends of the daughter chromosomes soon begin to converge toward the two endosomes (pl. 29, fig. 17) formed by the constriction and division of the original one. This stage is followed by the constriction of the two daughter nuclei at the end of the anaphase.

In the anaphase and telophase there have been observed in a number of instances (pl. 29, fig. 19; pl. 30, fig. 21) cytoplasmic structures at the end of the two daughter nuclei; these are similar to the "polar caps" described by Dunkerley (1921). Whether these caps are of any significance in mitosis is not certain, but it seems very probable that these cytoplasmic areas are comparable with the "centrospheres" described by Chatton (1914) in living specimens of *Blastodinium crassum*, with the spheres of *Noctiluca* (Calkins, 1899), and possibly with the centrospheres of echinoderm eggs (Chambers, 1917).

THE TELOPHASE

In the telophase the nuclear membrane has parted in the middle, giving rise to two pear-shaped daughter nuclei with their endosomes extending toward the margins which have just been separated (pl. 30, fig. 20); the nuclear membranes at first retain the shape of the anaphase figure to some extent, but as the endosomes move in toward the centers of the nuclei and the chromosomes begin to lose their parallel arrangement, the nuclear membrane comes to adhere more closely to the spherical mass of chromosomes (pl. 30, figs. 21, 22).

In the late telophase (pl. 30, fig. 24), the chromosomes are no longer parallel, but resemble the early prophase nuclei (pl. 26, fig. 3), in that the chromosomes are somewhat coiled. The centrosomes and flagella of both schizonts now occupy approximately their definitive positions, after the anterior schizont's centrosome-complex has migrated posteriorly from its position in the earlier telophase.

FISSION

My observations agree with the statement of Senn (1911) that, in all flagellates showing any indications of fission, two sets of flagella are already present. As shown above, outgrowth of two new flagella occurs in the late prophase (pl. 27, fig. 7). During the metaphase (pl. 27, fig. 8) elongation of the body begins. In the early anaphase (pl. 29, fig. 17) a constriction appears on one side near the middle of the body, marking the plane of division. The flagella of the anterior daughter soon become extended from a deeper region of this constriction on the left side of the body (pl. 29, fig. 17). As the nucleus reaches the telophase stage (pl. 30, figs. 20, 21), the groove around the body becomes deepened, and there appears on the left side an evagination which marks the beginning of the new tentacular lobe (pl. 30, figs. 21–24).

The anterior schizont gradually assumes the characteristic shape and structure as fission nears completion, and finally two new organisms are formed when the hypocone of the anterior daughter separates from the epicone of the posterior one. According to Senn (1911), this entire process of fission lasts for a little more than two hours.

DISCUSSION

NEUROMOTOR SYSTEM

The neuromotor system of a typical flagellate consists essentially of one or more flagella, each ending in a blepharoplast, and a rhizoplast connecting each blepharoplast with the extranuclear centrosome. Such a system has been established in the following groups of flagellates: *Distomatina* (Kofoid and Christiansen, 1915*a*, *b*, *c*), *Polymastigina* (Kofoid and Swezy, 1915*a*, *b*; Swezy, 1915*a*, *b*), *Hypermastigina* (Kofoid and Swezy, 1919*b*, *c*), *Euglenoidina* (Baker, 1921; Hall, 1923), and also in two members of the *Dinoflagellata*, *Oxyrrhis marina* and *Ceratium hirundinella* (Hall, MSS).

The first workers to apply the name 'neuromotor system' to the fibrillar structures of flagellates, and to emphasize such a system as a fundamental characteristic of flagellate protozoa, were Kofoid and his students (Kofoid, 1916; Kofoid and Swezy, 1915*a*, *b*; Kofoid and Christiansen, 1915*a*, *b*). Since their early work on the Polymastigina and Distomatina, Kofoid and Swezy, in addition to extending their observations to other members of these groups, have demonstrated neuromotor systems in several of the *Hypermastigina* (Kofoid and Swezy, 1919*b*, *c*).

The neuromotor system of the *Euglenoidina* was first worked out by Baker (1921) in the Protozoology Laboratory of Emory University. Baker has found that the neuromotor system of *Euglena* is composed of a flagellum ending in a blepharoplast, which is joined by a rhizoplast to a granule just outside the nuclear membrane; the behavior of this granule in mitosis is comparable with that of the extranuclear centrosome in other flagellates. In an investigation begun at Emory University, the author has described a similar neuromotor system in *Menoidium incurvum* (Hall, 1923) and, incidentally, has been able to verify the earlier observations of Baker on *Euglena*. In addition, a similar system has been discovered in *Heteronema acus* by Rhodes and Kirby (MSS), also of Emory University.

The present paper demonstrates such a neuromotor system in *Oxyrrhis marina;* and in another dinoflagellate, *Ceratium hirundinella* (fig. G, Hall, MSS) flagellar rhizoplasts and an extranuclear granule, apparently homologous with the extranuclear centrosome of *Oxyrrhis,* have been found. Jollos (1910), in several of his figures of *Gymnodinium fucorum,* shows clearly that he has found similar structures in this dinoflagellate. It is unfortunate that Jollos failed to work out more in detail the behavior of the neuromotor apparatus in mitosis, for he figures blepharoplasts, flagellar rhizoplasts extending from the blepharoplasts to an extranuclear granule, and also another rhizoplast connecting this extranuclear granule with the endosome. Apparently he has found blepharoplasts, flagellar rhizoplasts, extranuclear centrosome, and intranuclear rhizoplasts; from his figures, it seems that the neuromotor system of *Gymnodinium* is very much like that of *Oxyrrhis.*

In a brief paper, Awerinzew (1906–1907) figures for *Chilomonas paramecium* structures which suggest that *Chilomonas* probably has a typical neuromotor system. Nägler (1912) also describes such a fibrillar system in *Chilomonas;* he figures flagella ending in blepharoplasts, with rhizoplasts connecting the blepharoplasts with the nuclear membrane. Bělař (1916) likewise describes such a system in *Chilomonas;* in some of his figures he shows typical rhizoplasts extending to the nucleus and ending in granules on the nuclear membrane.

THE PARADESMOSE

The paradesmose, a characteristic morphological feature of mitosis in flagellates, was first described by Kofoid and Swezy (1915*a*) in *Trichomonas.* This structure is a more or less delicate fibril drawn out between the daughter centrosomes or centroblepharoplasts as they separate at the beginning of binary fission. Two different types of paradesmoses have been described. The first type, which might be termed the *'centroblepharoplast-paradesmose,'* connects the two daughter centroblepharoplasts as they draw apart in mitosis. A paradesmose of this type has been described in *Trichomonas* (Kofoid, 1916; Kofoid and Swezy, 1915*a*), *Menoidium incurvum* (Hall, 1923), *Euglena* (Hall, 1923); in addition, Dobell (1908) apparently has figured such a paradesmose in *Copromonas subtilis,* but did not discuss its significance.

The second type, the *'centrosome-paradesmose'* was first described by Kofoid and Christiansen (1915*a, b*) in *Giardia muris.* Since then it has been described in a number of different flagellates, including *Trichomitus termitidis* (Kofoid and Swezy, 1919*a*), *Leidyopsis sphaerica* (Kofoid and Swezy, 1919*c*), *Trichonympha campanula*

(Kofoid and Swezy, 1919 *b, d*), ***Chilomastix mesnili*** (Kofoid and Swezy, 1920), ***Giardia enterica*** (Kofoid and Swezy, 1922), ***Ditrichomonas termitis*** (Cutler, 1919), ***Collodictyon triciliatum*** (Rhodes, 1919), ***Hexamitus ovatus*** (Swezy, 1915*a*), ***Trichomitus parvus*** (Swezy, 1915*b*), ***Dinenympha*** (Kirby, MSS). The paradesmose of ***Oxyrrhis*** is also of this type. In addition, Nägler (1912) has figured (pl. 13, fig. 32), a fibril connecting the two extranuclear granules in a stage corresponding, apparently, to the anaphase. This structure figured by Nägler appears to be homologous with the centrosome-paradesmose, as described above for other flagellates.

So far, the presence of a paradesmose has been determined in the following groups of flagellates: *Distomatina, Hypermastigina, Polymastigina, Euglenoidina, Dinoflagellata (Oxyrrhis)*, and probably also in the *Cryptomonadina.*

Kofoid and Swezy (1921) consider the 'sphere' of *Noctiluca* (Calkins, 1899) as a structure analogous to the centrosome-paradesmose of other flagellates. Since this sphere, or amphiaster, is essentially an extranuclear 'thread' (highly specialized in this case) connecting the daughter extranuclear centrosomes, such an interpretation seems quite justifiable.

THE INTRANUCLEAR RHIZOPLAST

An intranuclear rhizoplast for dinoflagellates seems to have been described previously by Jollos (1910) in *Gymnodinium fucorum;* in his paper (pl. 8, figs. 26–28, 31), Jollos figures rhizoplasts extending from granules just outside the nuclear membrane to the endosome inside the nucleus. These rhizoplasts figured by Jollos resemble that found in *Oxyrrhis*, except that two intranuclear rhizoplasts in one nucleus have never been observed in this flagellate. Intranuclear rhizoplasts have likewise been found in flagellates belonging to other orders. Awerinzew (1906–1907) figures, but does not name such a structure in *Chilomonas paramecium.* The intranuclear rhizoplast has also been described in several species of *Giardia* (Kofoid, 1916; Boeck, 1917; Kofoid and Swezy, 1922). Kofoid (1916) suggests that the connection of the neuromotor apparatus, by the intranuclear rhizoplast, to the karyosome in the nucleus of *Giardia* indicates "a fundamental structural and functional relation between the nucleus and this complex of extranuclear organelles."

Boeck (1917) seems to have shown that there is some connection between the intranuclear rhizoplast and the early polarization of the chromatin material within the nucleus of *Giardia.* A similar polarization of the chromosomes in *Oxyrrhis* suggests that a similar relationship may exist also in this flagellate. Boeck apparently found no splitting stages in his observations on the intranuclear rhizoplast of *Giardia,* and it has also been impossible to find such a stage in *Oxyrrhis.* Rhodes (1919), however, has found an intranuclear rhizoplast, in *Collodictyon triciliatum,* which seems to split at the beginning of mitosis.

The persistence of this intranuclear rhizoplast in some of the flagellates might be accounted for on the basis of Minchin's (1912) theory of the phylogenetic origin of the different types of flagellar attachments. Minchin suggests that, originally, the ancestors of the present flagellates were non-flagellated organisms, with a simple nucleus containing a centriole. Then, in its origin, the first flagellum was a direct outgrowth from the centriole of the nucleus. By assuming that, in the next stage, the extranuclear centrosome is formed on the flagellum just outside the nuclear membrane, replacing the intranuclear centriole, while the intranuclear portion of the flagellum (connecting the extranuclear centrosome with the endosome) persists, the presence of an intranuclear rhizoplast in *Oxyrrhis* and other flagellates might be accounted for. As Kofoid (1916) points out, Wilson's (1916) observations on the life history of *Naegleria gruberi,* a soil amoeba, support in general this theory of Minchin (1912). In assuming the flagellate stage, this amoeba passes through the hypothetical changes outlined by Minchin. A fibril grows out from the karyosome of the nucleus and extends to the nuclear membrane as an intranuclear rhizoplast. A granule appears on the outside of the nuclear membrane, at the tip of this rhizoplast; outgrowth of the rhizoplast is continued, and the granule is pushed out into the cytoplasm to form the blepharoplast of the flagellum. The intranuclear portion of the flagellum, the 'intranuclear rhizoplast,' persists, resembling that found in such flagellates as *Oxyrrhis, Gymnodinium, Giardia, Chilomonas,* and *Collodictyon.*

MITOSIS

The characteristics of mitosis in *Oxyrrhis* may be summarized as follows:

1. The nuclear membrane persists throughout the process of mitosis, as is the case in most other flagellates.

2. The nucleus contains an endosome which elongates in the metaphase, and divides by constriction in the anaphase.

3. The chromosomes take the form of strings of chromomeres; these chromomeres seem to persist throughout the life cycle of the flagellates. So far as can be determined, the endosome bears no structural relation to chromosome formation.

4. Longitudinal splitting of the chromosomes begins in the late prophase; the split begins at one end of each chromosome and proceeds toward the other, forming V-shaped structures.

5. An 'equatorial plate' phase is produced by the unfolding of the V-shaped structures formed by the progressive unipolar splitting of the chromosomes; a densely staining 'cylinder' of parallel chromosomes is thus formed in the late metaphase.

6. An extranuclear centrosome is present; this structure divides at the beginning of binary fission. An intranuclear rhizoplast, connecting the centrosome with the endosome, has been observed in a number of cases.

7. A centrosome-paradesmose is formed between the two daughter centrosomes as they draw apart in the prophase; this structure does not disappear until the late anaphase.

8. In view of the ultimately transverse fission of *Oxyrrhis*, it is interesting to note that mitosis begins as in the Euflagellata, just as if in preparation for a longitudinal fission. This relict phase of ordinary flagellate mitosis soon passes, however, and the nucleus and neuromotor apparatus become shifted to the position required for the impending transverse division.

In its general aspects, nuclear division in *Oxyrrhis* is similar to that in euglenoids (Hall, 1923). In both cases an endosome is present and behaves similarly; in both the euglenoids and *Oxyrrhis* a paradesmose is present; and likewise, the division center in each case is an extranuclear centrosome. Splitting of the chromosomes is longitudinal in both, the process taking place by a progressive unipolar splitting of each chromosome, such that the separation of the free ends of the daughter chromosomes results in the formation of V-shaped and U-shaped structures. In both, the unfolding of the V's produces a belt of chromosomes surrounding the elongated endosome during the metaphase.

Calkins (1899) describes, in *Noctiluca,* a process of mitosis which resembles more closely that in *Oxyrrhis.* In the prophase he figures a spireme stage very similar to that of *Oxyrrhis,* and also that of *Ceratium hirundinella* (Hall, MSS). Following the spireme stage, the chromosomes split longitudinally in a progressively unipolar division, and the separated ends of the daughter chromosomes move apart toward the poles of the amphiaster to form an equatorial plate. The plate then divides in the equatorial plane, forming the daughter nuclei.

THE ENDOSOME

From the published descriptions, there seem to be few structures in dinoflagellates strictly comparable with the endosome of *Oxyrrhis. Gymnodinium fucorum* (Jollos, 1910) exhibits a similar endosome, which also divides in the anaphase, behaving much as does the one in *Oxyrrhis.* Similar bodies in the nuclei of many other flagellates seem to be either *karyosomes* or *plasmosomes* (Agar, 1920). If *karyosomes,* they are composed of chromatin which is used up in the formation of the chromosomes. If *plasmosomes,* they do not contain chromatin, but disappear nevertheless in mitosis; hence, their substance probably plays a part in the cyclic changes of the chromatin in the nucleus. In the case of the endosome of *Oxyrrhis,* this is not the case; this structure persists throughout the process of mitosis, and instead of undergoing a loss of its substance it seems rather to increase in size prior to its division in the anaphase.

As was pointed out by Schaudinn (1896), this endosome of *Oxyrrhis* is strikingly similar to a corresponding structure described in a number of the euglenoids; Schaudinn, however, calls this endosome a "Nucleolo-Centrosoma." He seems to have misinterpreted a small food mass as an endosome which had migrated out of the nucleus in *Oxyrrhis,* and on this evidence is based his theory that the primitive position of the "Nucleolo-Centrosoma" is outside, and not inside the nucleus of flagellates. If only that part of Schaudinn's theory which deals with the primitive position of the centrosome be considered, his conception is in accord with much of the later work on flagellates, in which the presence of an extranuclear centrosome has been found to be the rule; on the other hand, there is no evidence that the endosome

(Schaudinn's "Nucleolo-Centrosoma") has migrated into the nucleus from a primitively extranuclear position. Furthermore, the demonstration of an extranuclear division-center in euglenoids (Baker, 1921; Hall, 1923) and *Oxyrrhis,* forms which Schaudinn believed to possess nucleolo-centrosomes, affords sufficient evidence for discrediting Schaudinn's theory.

It seems then that the endosome of *Oxyrrhis* is a non-chromatic body which apparently plays no part in the formation of the chromosomes, and which is in no sense to be considered a nucleolo-centrosome. Its connection with the extranuclear centrosome by means of an intranuclear rhizoplast suggests that the endosome may serve as a sort of central spindle in the division of the nucleus. The similarity, in relation to the chromosome belt of the metaphase, between the endosome of *Oxyrrhis* and the amphiaster of *Noctiluca* also supports such an hypothesis; this amphiaster of *Noctiluca* (Calkins, 1899) becomes almost completely surrounded by the metaphase belt of chromosomes. It should be pointed out, however, that the amphiaster of *Noctiluca* remains outside the nuclear membrane, while the endosome of *Oxyrrhis* is always inside the nucleus.

THE CENTRIOLE

As is the case in much of the older work on flagellates, a centriole has also been described in several of the dinoflagellates. Keysselitz (1908) figures a granule in the endosome of *Oxyrrhis,* and states that the process of nuclear division begins in the 'karyosome,' which functions as a division-center; the centriole of the 'karyosome' divides, and its division products separate, initiating mitosis. The presence of such a granule could not be verified in my material; the behavior of the endosome itself indicates that it is a comparatively inert structure in mitosis, and it appears to undergo no change whatever in size or structure until some time after the extranuclear centrosome has divided in the early prophase. Senn (1911) was unable, also, to find a centriole in his material; and in addition, Dunkerley (1921) in his recent paper on *Oxyrrhis* denies the presence of a centriole in the endosome.

Jollos (1910) has also described a centriole in the endosome of *Gymnodinium fucorum,* and his figures clearly indicate the presence of a granule; however, until his observations have been verified or disproved by other workers, his description can hardly be admitted as evidence. Hence, as is the case with the descriptions of a centriole in other groups of flagellates (notably the euglenoids, Hall, 1923), the cytological evidence in favor of the centriole in the endosome of dinoflagellates seems to be inadequate.

CYTOPLASMIC CHANGES DURING MITOSIS

Chambers (1917), in his study of the living eggs of various marine animals, has found that the process of mitosis is intimately connected with certain physical changes in the cytoplasm immediately surrounding the nucleus; a periodic reversal of the cytoplasm from the sol to the gel state and vice versa occurs during cell division. The "sphere" (containing the centrosomes), in the cells studied, is described as a "liquid region free from granules" near the nucleus. With the formation of the amphiaster this clear area of the cytoplasm comes to surround the nucleus, which then lies in a mass of less dense cytoplasm (in the sol state).

The phenomena observed in metazoan eggs as he describes them are similar to the behavior of the cytoplasm observed in *Oxyrrhis*. In the prophase a clear, or less densely stained area of cytoplasm becomes evident around the nucleus (pl. 26, fig. 3). This clear area persists throughout the metaphase (pl. 27, figs. 8, 9; pl. 28, figs. 11, 12, 14) and anaphase (pl. 29, figs. 15, 19); in the telophase, a similar clear area surrounds each daughter nucleus (pl. 30, figs. 21–24). These clear cytoplasmic areas are also figured by Dunkerley (1921) from the prophase to the telophase. In addition, Dunkerley describes more condensed areas at the poles of the nucleus—his "polar caps," which I have also observed (pl. 29, fig. 19). Chatton (1914) has described in *Blastodinium crassum* the appearance of centrospheres at the poles of the nucleus; these appear to be homologous with the "polar caps" of *Oxyrrhis* (Dunkerley, 1921), and also with the centrospheres of metazoan cells observed by Chambers (1917). The behavior of the "sphere" in *Noctiluca* (Calkins, 1899) also simulates that of the metazoan centrosphere. Hence, it seems probable that, on the basis of existing morphological descriptions, mitosis in at least some of the dinoflagellates is associated with, and dependent upon, changes in the consistency of the cytoplasm surrounding the nucleus. The centrospheres, polar caps, or amphiasters are probably developed around the extranuclear centrosomes of these flagellates just as in metazoan cells the asters develop around the centrosomes (also extranuclear).

SUMMARY

1. *Oxyrrhis marina* is a primitive dinoflagellate, belonging to the family Protodiniferidae of the order Diniferidea (Kofoid and Swezy, 1921). It possesses the following characteristics peculiar to dinoflagellates: girdle, sulcus, tentacular lobe, longitudinal and transverse flagella, and persistently beaded chromosomes.

2. The organisms have been observed both in San Francisco Bay and Monterey Bay, on the California coast.

3. The flagellates have been grown successfully in laboratory aquaria in various culture media.

4. A neuromotor system is described in *Oxyrrhis,* consisting of the two flagella with their blepharoplasts and flagellar rhizoplasts, an extranuclear centrosome and an intranuclear rhizoplast.

5. A centrosome-paradesmose is described.

6. Division of the chromosomes occurs by unipolar longitudinal splitting; as the daughter chromosomes move apart, they unfold to form an equatorial belt of chromosomes surrounding the elongated endosome.

7. The nucleus contains an endosome which persists throughout mitosis, and resembles in behavior the homologous structure in euglenoids.

8. The beaded structure of the chromosomes seems to persist during the entire life cycle of the organisms.

9. Fission appears to begin as if in preparation for ordinary longitudinal division; later the nucleus and neuromotor apparatus become shifted so that the paradesmose lies parallel to the major axis of the body.

10. There is some evidence that mitosis is accompanied by changes in the consistency of the cytoplasm, resembling the sol-gel phenomena observed by Chambers in metazoan cells.

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EXPLANATION OF PLATES

PLATE 26

Oxyrrhis marina. × 2340.

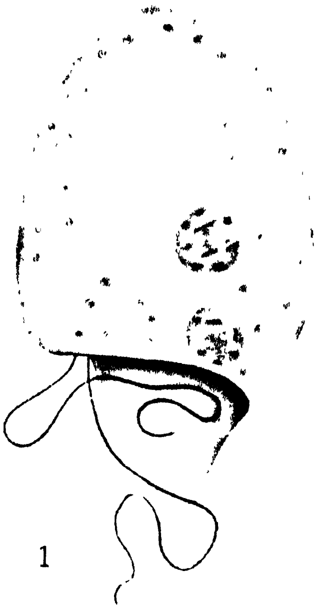
Fig. 1. Camera lucida sketch of living *Oxyrrhis* viewed from dorsal side, showing girdle, flagella, food bodies, and numerous oil globules; the clear oval area marks the position of the nucleus.

Fig. 2. Camera lucida sketch of living flagellate from ventral side; note nucleus (shown diagrammatically) with beaded chromosomes, sulcus, girdle, and tentacular lobe.

Fig. 3. Ventral view; optical section through nucleus in prophase; radial arrangement of chromosomes not yet established. Note short paradesmose. Bordeaux red, followed by iron haematoxylin.

Fig. 4. Nucleus in about the same stage as that shown in figure 5 (early prophase), with chromosomes stained more distinctly; several small food masses in endoplasm; body viewed from left side. Iron haematoxylin and Congo red.

Fig. 5. Nucleus shown in transverse section; note radial arrangement of beaded chromosomes around endosome in early prophase. A short paradesmose is present. Bordeaux red followed by iron haematoxylin.



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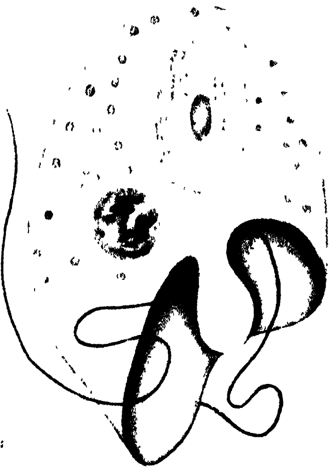

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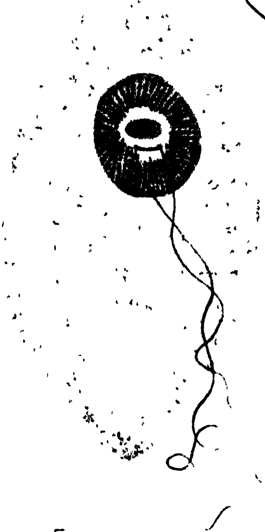

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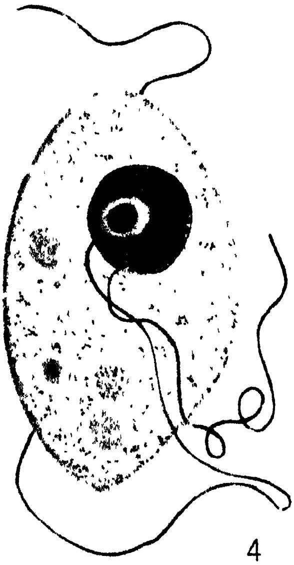

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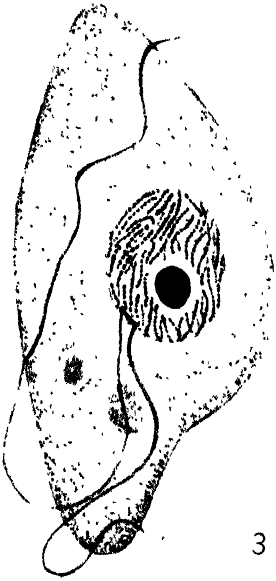

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PLATE 27

Oxyrrhis marina. × 2340.

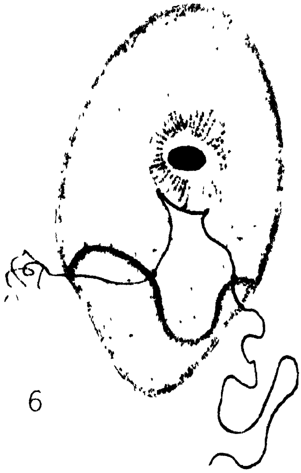
Fig. 6. Organism viewed from left side. Note the two blepharoplasts and centrosomes and the short paradesmose. Nucleus in prophase. Bordeaux red followed by iron haematoxylin.

Fig. 7. Ventral view; two pairs of flagella present at the end of the prophase. Note radial arrangement of chromosomes at the pole of the nucleus, and the short fibril (intranuclear rhizoplast) extending from one of the centrosomes to the endosome. Bordeaux red followed by iron haematoxylin.

Fig. 8. Dorsal view; early metaphase. The nucleus has shifted so that its long axis is now parallel to that of the body. Note chromosomes in process of unfolding to form the equatorial plate. Bordeaux red followed by iron haematoxylin.

Fig. 9. Ventral view; early metaphase; frontal section through nucleus. Note long paradesmose, and fibril extending from one centrosome to the endosome. Bordeaux red followed by iron haematoxylin.

Fig. 10. Dorsal view; late metaphase. Note elongated endosome, and chromosomes in the 'equatorial plate.' The anterior pair of flagella extend under the nucleus; a paradesmose could not be determined. Iron haematoxylin.



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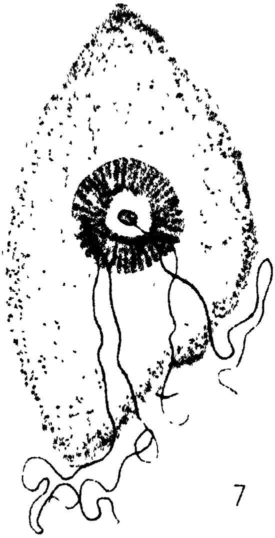

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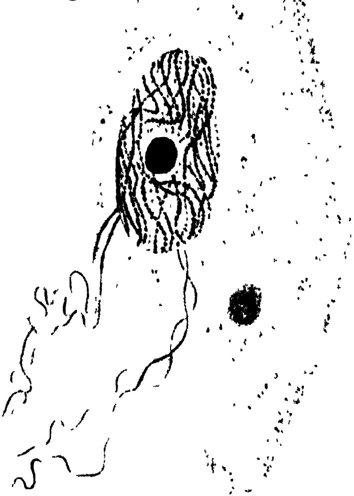

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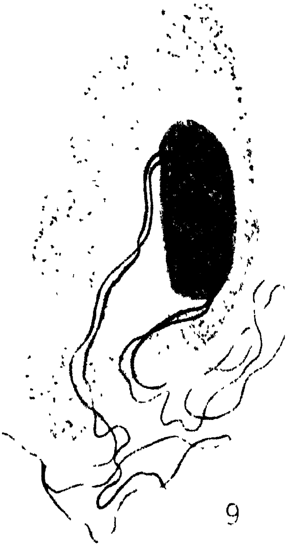

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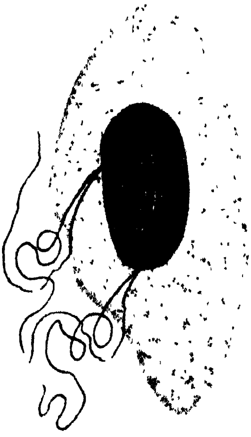

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PLATE 28

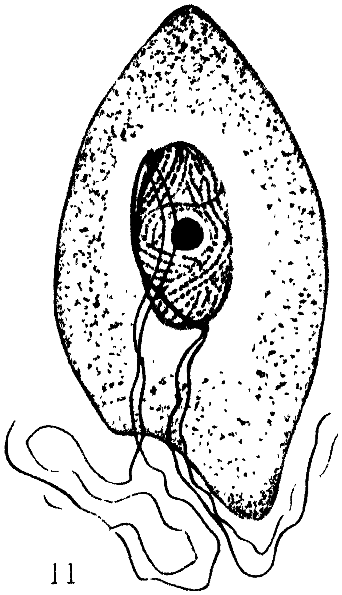
Oxyrrhis marina. × 2340.

Fig. 11. Dorsal view; metaphase at about same stage as shown in figure 9. Note long paradesmose, and chromosomes in process of unfolding. Bordeaux red followed by iron haematoxylin.

Fig. 12. Organism viewed from right side; nucleus in metaphase at about the same stage as that shown in figure 11. Note unfolding chromosomes, and fibril extending from posterior centrosome to the endosome. Iron haematoxylin and Congo red.

Fig. 13. Dorsal view; early metaphase, frontal section through nucleus. Note at anterior end of nucleus the clear hyaline area in which lies the centrosome. Bordeaux red followed by iron haematoxylin.

Fig. 14. Dorsal view; fairly late metaphase. Note abnormal constriction of the endosome, probably caused by the constriction of the nucleus. Anterior flagella extend under the nucleus. Bordeaux red followed by iron haematoxylin.



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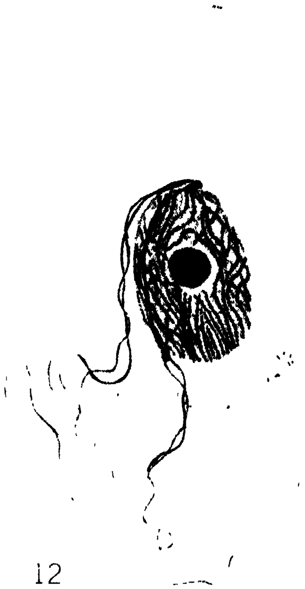

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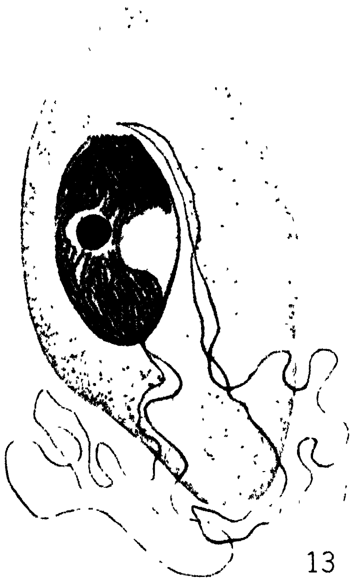

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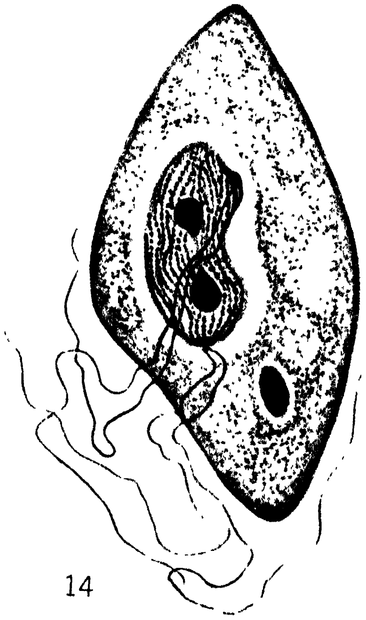

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PLATE 29

Oxyrrhis marina. × 2340.

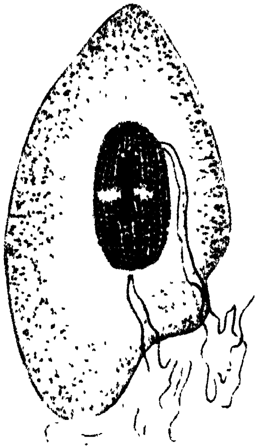
Fig. 15. Ventral view; early anaphase. Note almost complete separation of daughter chromosomes in the equatorial plane. The anterior pair of flagella extend under the nucleus. Iron haematoxylin and Congo red.

Fig. 16. Ventral view; later anaphase. Daughter chromosome plates completely separated; note elongated endosome. Anterior flagella extend under the nucleus. Iron haematoxylin and Congo red.

Fig. 17. Ventral view; later anaphase. Ends of daughter chromosomes have converged toward poles of the elongated endosome. Paradesmose and anterior flagellar rhizoplasts extend under the nucleus. Note depression on the side, from which the anterior flagella extend. Iron haematoxylin and Congo red.

Fig. 18. Dorsal view; late anaphase. The endosome has divided, and the daughter halves have migrated into the center of the chromosome belts; the nuclei are shown in surface view. Iron haematoxylin and Congo red.

Fig. 19. Dorsal view; later anaphase. Fission is more advanced. The nuclei are shown in optical sections. Iron haematoxylin and Congo red.



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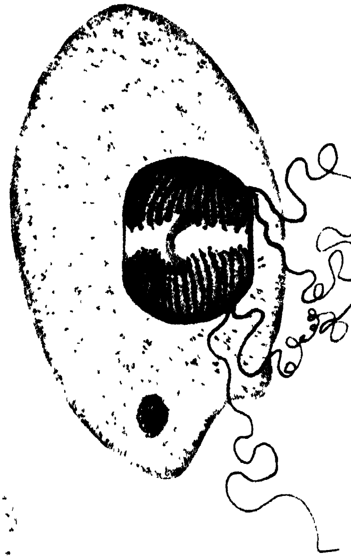

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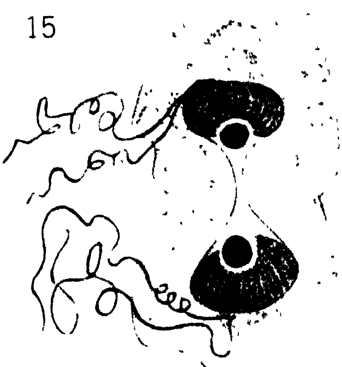

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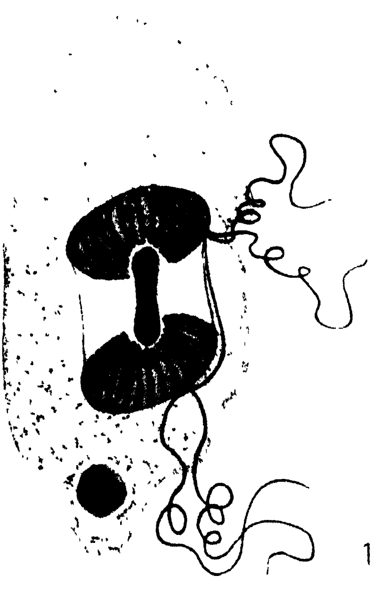

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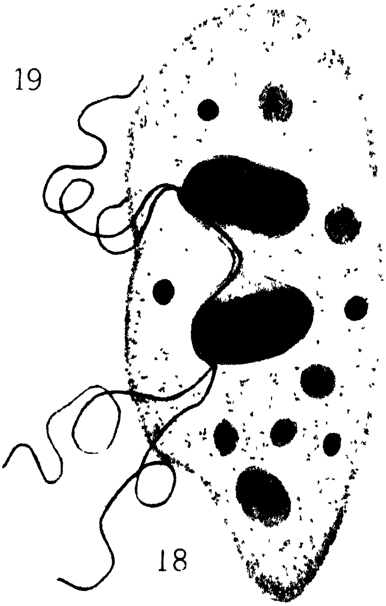

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PLATE 30

Oxyrrhis marina. × 2340.

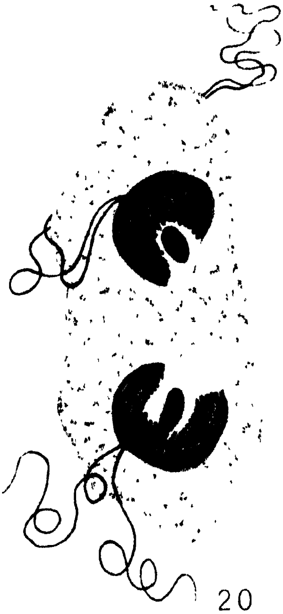
Fig. 20. Dorsal view; early telophase. Note shape of daughter nuclei, which are shown in optical section. Iron haematoxylin and Congo red.

Fig. 21. Dorsal view; later telophase. The nuclei have rounded up, but the chromosomes are still arranged in parallel rows. Note "polar caps" (Dunkerley, 1921) at ends of nuclei. The anterior flagellar rhizoplasts extend under the nucleus. Note hyaloplasmic areas around the daughter nuclei. Iron haematoxylin.

Fig. 22. Ventral view; telophase. The centrosome of the anterior schizont has migrated to its final position. Nuclei shown in optical section; chromosomes still in parallel rows. The tentacular lobe of the anterior daughter has appeared. Bordeaux red followed by iron haematoxylin.

Fig. 23. Ventral view; telophase, at about same stage as figure 22; nuclei shown in surface view. Iron haematoxylin and Congo red.

Fig. 24. Ventral view; later telophase, fission almost completed. Nuclei shown in surface view; parallel arrangement of chromosomes is lost. Iron haematoxylin and Congo red.



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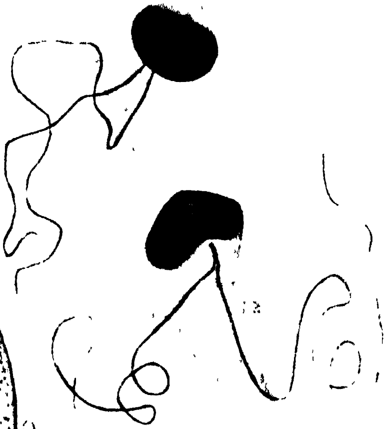

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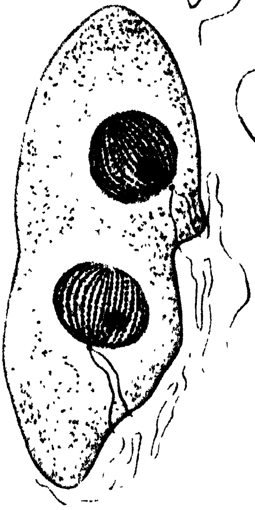

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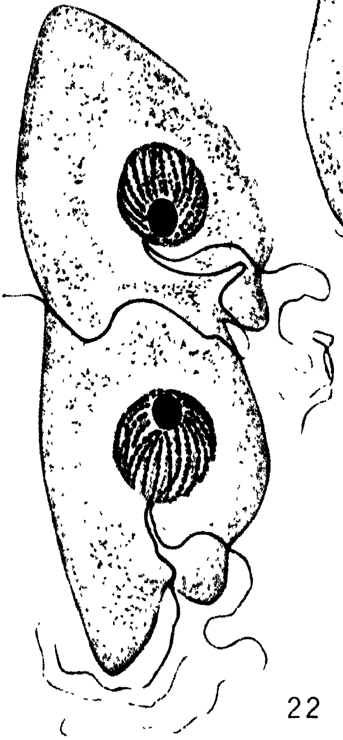

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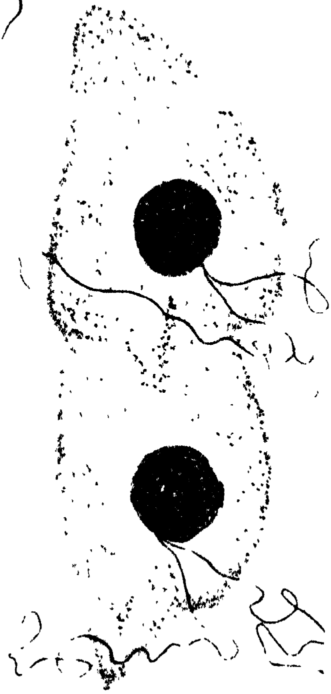

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A LARVAL ACANTHOCEPHALID, CENTRORHYNCHUS CALIFORNICUS SP. NOV., FROM THE MESENTERY OF HYLA REGILLA

and

MEGALODISCUS RANOPHILUS SP. NOV., A TREMATODE FROM THE RECTUM OF RANA PIPIENS

BY

RAYMUND MILLZNER

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ZOOLOGY
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A LARVAL ACANTHOCEPHALID, CENTRORHYNCHUS CALIFORNICUS SP. NOV., FROM THE MESENTERY OF HYLA REGILLA

BY

RAYMUND MILLZNER

North American Amphibia appear to be singularly free from Acanthocephali. Only one instance of infection with the adult parasites has been reported. Seven worms, later identified by Van Cleave as *Acanthocephalus ranae,* were found by Hassall in the intestine of a specimen of *Diemyctylus viridescens.* No larval Acanthocephali have been found in North American Amphibia, although two species, *Corynosoma semerne* and *Centrorhynchus aluncoris,* are common in European Amphibia.

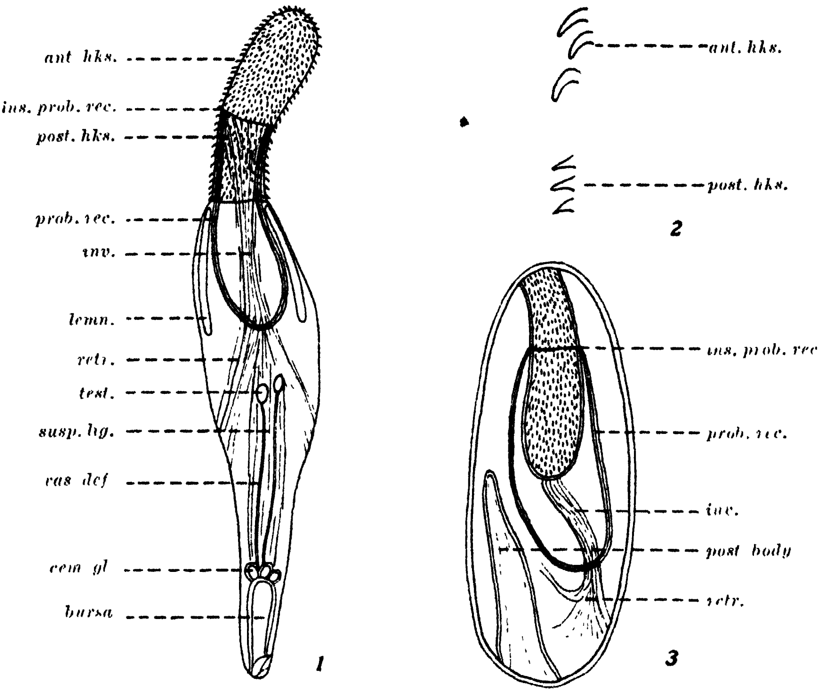
The genus *Centrorhynchus* is found in its mature stages in the intestine of birds. Although common in Europe, only a single female specimen belonging to a new species, *Centrorhynchus spinosus* (Van Cleave, 1916), is known from North America. It was found in the intestine of an egret, *Herodias egretta.*

The finding of larval Acanthocephali belonging to the genus *Centrorhynchus* in Pacific Coast tree frogs, *Hyla regilla,* is therefore of peculiar interest. The frogs were caught during March around the banks of a small pond near the head of Diamond Cañon, Oakland. Out of 86 frogs examined, 4, or 4.6 per cent, were infested with larval Acanthocephali. Numerous *Diemyctylus torosus* were also present in the same pool but none of the 22 examined contained Acanthocephali. The Acanthocephali were all encysted in the mesentery near the junction of the stomach and duodenum. Only one was found in each of the four infected frogs.

Centrorhynchus californicus sp. nov.

The encysted parasites are enclosed in a thick connective tissue capsule. After removal from the cyst they average 1.65 by 0.57 mm. The proboscis receptacle and proboscis as well as the posterior third of the body are invaginated into the middle portion of the body. When the encysted parasites were allowed to remain in warm slightly acidulated saline for a few minutes they evaginated the proboscis and posterior end.

After evagination, the average length is 3.15 mm. The width varies from 0.14 mm. at the posterior end to 0.38 mm. near the middle of the body. The proboscis is closely set with numerous hooks. The length of the proboscis is 0.88 mm.; the width, 0.20 mm. A slight constriction marks the insertion of the proboscis receptacle a little behind the middle of the proboscis. The length of the proboscis anterior to this insertion is 0.56 mm.; posterior to the insertion, 0.32 mm. The hooks are arranged in 43 transverse rows, of which 27 are anterior and 16 posterior to the insertion of the proboscis receptacle. There are about 24 hooks in each row. The hooks are of two distinct types. Those anterior to the insertion of the proboscis receptacle are rather large, 0.042 mm. long, and are distinctly recurved. Those posterior to the insertion are smaller, 0.031 mm. long, and are rose-thorn shaped. The hooks at the tip of the proboscis are slightly larger, 0.052 mm. long, than the others. The proboscis is separated from the body proper by a



Figs. 1–3. *Centrorhynchus californicus* sp. nov., from mesentery of *Hyla regilla*. *ant. hks.*, anterior proboscis hooks; *bursa*, copulatory bursa; *cem. gl.*, cement gland; *ins. prob. rec.*, insertion of proboscis receptacle; *inv.*, invertors of proboscis; *lemn.*, lemniscus; *post. body*, posterior portion of body; *post. hks.*, posterior proboscis hooks; *prob. rec.*, proboscis receptacle; *retr.*, retractors of proboscis receptacle; *susp. lig.*, suspensory ligament; *test.*, testis; *vas def.*, vas deferens.

Fig. 1. Larval form after evagination of proboscis and posterior portion of body. × 30.

Fig. 2. Hooks from proboscis. × 100.

Fig. 3. Encysted larval form. × 35.

slight rim. The proboscis receptacle consists of a two-layered, cylindrical muscular sac. It measures 0.94 by 0.36 mm. The invertors of the proboscis pass through the posterior tip of the proboscis receptacle and are then continued as retractors of the proboscis receptacle. The lemnisci are 0.54 mm. long. The two testes are small and undeveloped. They are oval, 0.056 by 0.040 mm., and lie imbedded in the large suspensory ligament. A narrow vas deferens passes posteriorly from each. The cement glands are three in number and are also small and undeveloped. The reproductive organs of the females cannot be definitely distinguished. No spines are present on the body.

This form differs from the only described North American member of the genus, *Centrorhynchus spinosus,* in the larger size of the proboscis, the greater number of rows of hooks, the larger size of the hooks, and in the relative sizes of the hooks on different portions of the probascis. Since the proboscis and hooks are well developed, and since the identification and differentiation of species within a genus is usually based on these characters, no difficulty would be met with in identifying the mature form of this parasite. The name *Centrorhynchus californicus* is proposed, therefore, for this form.

I wish to express my sincere appreciation to Dr. Charles A. Kofoid for his direction and aid during the study of this form.

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MEGALODISCUS RANOPHILUS SP. NOV., A TREMATODE FROM THE RECTUM OF RANA PIPIENS

BY

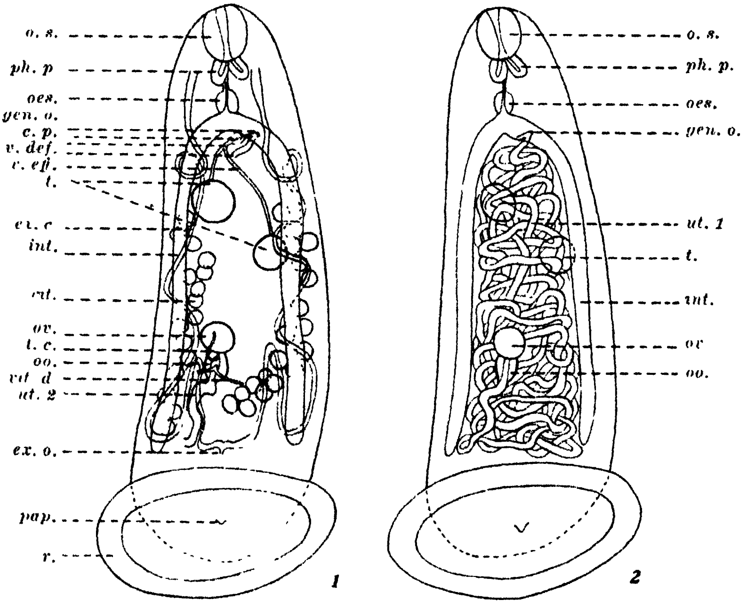
RAYMUND MILLZNER

Chandler (1923) described a new fluke from the rectum of *Amphiuma means* which he named *Megalodiscus americanus* and for which he created the genus *Megalodiscus.* This new genus belongs to the subfamily Diplodiscinae of Cohn (1904).

During an examination of some thirty *Rana pipiens* for parasites, three specimens of a fluke resembling *Megalodiscus americanus,* but differing from it in several important details, were found in the rectum near the cloaca. Not more than one fluke was found in one frog. All the flukes appeared to have passed the reproductive period since no eggs were present in the uterus although the organ was well developed and greatly convoluted. This condition of the flukes probably represented an approaching physiological limit to their vital activities, preceding their discharge from the host at the time of spawning. A similar discharge of intestinal parasites in *Rana virescens* during the spawning season is mentioned by Ward (1907). Two immature specimens of the same species were found in a *Rana pipiens* examined the previous summer.

Megalodiscus ranophilus sp. nov.

Description of mature specimens.—Body somewhat horn-shaped; in fresh material nearly circular in cross section. Length, 3.5–3.8 mm. Greatest width of body, 1.12 mm. just anterior to posterior sucker. Posterior sucker very large, 1.5 mm. in diameter, forming a shallow dish-like structure with a raised thickened rim. The center of the sucker bears a small papilla which is relatively more prominent in immature specimens. The oral sucker is large and almost globular in shape, 0.30 mm. in diameter, 0.32 mm. in length. The pharyngeal pockets are well developed, 0.19 mm. long. A fairly well defined oesophageal bulb is present at the junction of the oesophagus and the intestinal caeca. The intestinal caeca are unbranched and extend almost to the posterior sucker. The ovary is small, 0.21 by 0.18 mm., and is situated dorsally on the left side in the posterior part of the body. The ootype is immediately posterior to the ovary and is followed by a short, enlarged, and deeply-staining portion of the uterus. The uterus is coiled transversely and completely fills the space between the intestinal caeca. The uterus opens in common with the vas deferens ventral to and slightly to the right of the oesophageal bulb. The vitellaria consist of fifteen to twenty separate spherical follicles on each side, extending posteriorly from the level of the anterior testis almost to the posterior sucker. They are ventral to the intestinal caeca for most of their course, but posteriorly they turn medially and dorsally. The vitelline duct is transverse, posterior to the ovary, and opens into the ootype by a common duct. Laurer's canal opens dorsally at the level of the ovary. The testes are relatively small, two in number, ventral, and almost spherical. The anterior testis is on the left side; the posterior on the right. The anterior is 0.28 by 0.24 mm.; the posterior 0.23 by 0.21 mm. The vasa efferentia arise at the anterior ends of the testes, pass anteriorly, and join near the left intestinal caecum to form a vas deferens which, after passing into a small cirrus pouch, opens to the exterior in common with the uterus. Two large lateral excretory tubes, prominently marked out by dark concretions, are present. They parallel and wind around the intestinal caeca, each tube usually making two complete turns. The two longitudinal excretory tubes unite to form a slightly dilated transverse vessel posterior to the ovary and open just anterior to the posterior sucker in the middorsal line.



Figs. 1 and 2. *Megalodiscus ranophilus* sp. nov., from *Rana pipiens*. *c. p.*, cirrus pouch; *ex. c.*, excretory canal; *ex. o.*, excretory orifice; *gen. o.*, genital orifice; *int.*, intestine; *l. c.*, Laurer's canal; *oes.*, oesophagus; *oo.*, ootype; *o. s.*, oral sucker; *ov.*, ovary; *pap.*, papilla of posterior sucker; *ph. p.*, pharyngeal pocket; *r.*, thickened rim of posterior sucker; *t.*, testes; *ut. 1*, uterus; *ut. 2*, thickened portion of uterus following ootype; *v. def.*, vas deferens; *v. eff.*, vasa efferentia; *vit.*, vitellaria; *vit. d.*, vitelline duct.

Fig. 1. Dorsal view showing details of anatomy exclusive of the uterus. × 20.

Fig. 2. Dorsal view showing uterus. × 20.

COMPARISONS

This form differs from *Megalodiscus americanus* Chandler, the only other species in the genus, in several respects. The posterior sucker is relatively slightly smaller. The oral sucker is globular instead of oval and the pharyngeal pockets are smaller. The vitellaria, although about the same in number, extend almost twice as far anteriorly, reaching to the level of the anterior testis, instead of only to the posterior testis. The testes instead of being very large are rather small. Because of these differences, this form appears to be specifically distinct from *Megalodiscus americanus,* and the name *Megalodiscus ranophilus* is proposed for it.

The host, *Rana pipiens,* is widely distributed throughout the United States, east of the Sierras. The frogs from which these parasites were obtained came from a dealer in San Francisco and the locality at which they were captured could not be determined.

My sincere thanks are due Dr. Charles A. Kofoid for direction and advice during the preparation of this paper.

Zoological Laboratory,
University of California.

Transmitted September 22, 1924.

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ON THE NUMBER OF CHROMOSOMES AND THE TYPE OF MITOSIS IN ENDAMOEBA DYSENTERIAE

BY

CHARLES A. KOFOID AND OLIVE SWEZY

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ON THE NUMBER OF CHROMOSOMES AND THE TYPE OF MITOSIS IN ENDAMOEBA DYSENTERIAE

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CHARLES A. KOFOID AND OLIVE SWEZY

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INTRODUCTION

In our account of the occurrence of motile amoebas in the bone marrow in arthritis deformans of Ely's second type (Kofoid and Swezy, 1922*a*, *b*) and in the lymphatic glands in Hodgkin's disease (Kofoid, Boyers, and Swezy, 1922*a*, *b*), we based our determination of the amoebic nature of the cells which we called amoebas in part upon the type of mitosis and the number of chromosomes we have found in these cells.

In his review of our work on *Councilmania lafleuri* in the Tropical Diseases Bulletin, Wenyon (1921) dismisses our evidence of typical amoebic mitosis in that species based on our clearly figured instance of two nuclei in the metaphase (Kofoid and Swezy, 1921, pl. 20, fig. 13) with definite chromosomes and intradesmose, with the following:

> The *assertion* [italics ours] that the new amoeba has eight chromosomes during nuclear division, whereas *E. coli* has only six, comes as a surprise to the reviewer, who was under the *impression* [italics ours] that no definite chromosomes are formed during the division of the nucleus of *E. coli.* Such stages of the division of the nucleus of *E. coli* as he has seen give no indication of chromosome formation, and this is borne out by Dobell's detailed description of the division of the nucleus of *E. histolytica,* which is of a similar type.

In a review in the Tropical Diseases Bulletin of two of our papers (Kofoid and Swezy, 1922*a*, Kofoid, Boyers, and Swezy, 1922*a*), Dr. Manson-Bahr (1922, pp. 684–685) makes the following statement:

> Considerable hesitation will be felt in accepting the interpretation or conclusions of these workers without more direct evidence than that based upon mitotic figures. . . . Neither the argument nor the figures of individual "amoebas" in the affected gland are at all convincing.

It appears that these critics have been influenced in their statements largely by the account and figures of mitosis in *E. dysenteriae* in Dobell's (1919, pl. 3) "The Amoebae Living in Man" rather than by the evidence as to the morphology of mitosis in the Amoebina generally as available in the investigations of other protozoologists. Dobell's account deals with amoebas in sections of intestinal ulcers in kittens experimentally infected with amoebas from man. They are therefore comparable with our published figures in so far only as they deal with the motile forms *in tissues*. They differ in that they are from an alien host whose death rather quickly results from extensive ulceration of the colon and in which the normal type of encystment of the amoebas in the stools has not been found. This interruption in the normal life-history of the parasite is indicative of an unfavorable environment.

Dobell's figures contain two prophases (his figs. 43, 44), a single amphiaster or metaphase at approximately the formation of the equatorial plate (his fig. 45), two anaphases (his figs. 46–47) in which chromosomes are not to be expected in clearly defined form, and four telophases (his figs. 48–51). His single metaphase shows some evidence of the emerging and assembling chromosomes. Their number is uncertain since there are a number of grayish structures in addition to about six intact or parting darker bodies. The latter are presumably chromosomes. The former, judging from the optical section of the nuclear wall, may be vague images of peripheral chromatin. There is possibly a larger amount of this chromatin in the active dysenteric phase of the life of the amoeba than in amoebas from cases of chronic amoebiasis in man.

The limitation of Dobell's account to two atypical or incompletely analyzed prophases and one indistinct figure of a metaphase, while six later stages fill out his plate, naturally gives an impression of absence of definite chromosomes. But this account is incomplete and quite misleading as an adequate picture of the process of mitosis in *Endamoeba dysenteriae*. We present in this paper evidence bearing on the type of mitosis and number of chromosomes in amoebas or their cysts from the human host. Our material has enabled us to gain a clear picture of the process in its several phases.

Many of our most instructive preparations came from a single thermos bottle specimen (case 25573) of a fluid stool brought promptly to the laboratory. Smears were made from this at once. These smears contain both motile amoebas and cysts in the early stages of nuclear multiplication. Mononucleate and binucleate cysts abound in the preparations and some of them present nuclear spindles in favorable positions. The race is unfortunately a medium-sized one, most of the cysts having a diameter between ten and twelve microns. The glycogen vacuole in these cysts is usually very large, crowding the nuclei to the wall. The nuclei are often obscured by the unusually large number of chromatoidal bodies. A few stages are figured in this paper from seven other cases none of which yielded mitotic figures in an abundance comparable with that in case 25573.

THE MITOTIC PROCESS IN ENDAMOEBA DYSENTERIAE

The process of mitosis in *Endamoeba dysenteriae* is of the type normal to the parasitic Amoebina in all essential particulars. There is no unique peculiarity in the process at any phase. It conforms in each important structural feature and in the sequence of the behavior of the nuclear organelles to the form of mitosis to be expected in this group in the light of previous investigations (Mathis and Mercier, 1916, 1917; Nöller, 1922) as well as our own (Kofoid and Swezy, 1921; Swezy, 1922). It is likewise of the same type as that described by us in the motile forms in the tissues in arthritis deformans (Kofoid and Swezy, 1922*a*, *b*) of Ely's second type and in Hodgkin's disease (Kofoid, Boyers, and Swezy, 1922*a*, *b*).

We distinguish in this process the interphase with normal resting nucleus, the prophase in which the daughter centrosomes form and the chromosomes emerge and divide, the modified amphiaster in which the divided chromosomes assemble in the equatorial region of the spindle, the anaphase in which they migrate toward the poles, and the telophase in which the nucleus constricts into two which then return to the interphase.

This process can be followed in the mononucleate and binucleate cysts in which the nuclei are going through the first and second divisions, respectively, resulting in the four-nucleate cyst typical of

E. dysenteriae. Coincidently with this process of nuclear division profound metabolic changes are going on in the cytoplasm and in its metaplasmic contents which environ the nuclei. In the mononucleate cyst (pl. 31, figs. 5, 9) the reserve food material is accumulated in one large, subcentral, subspherical mass of glycogen whose diameter often exceeds half that of the cyst. In some instances (pl. 31, figs. 2, 7) the glycogen is diffused through the cytoplasm in a cloud of smaller, less sharply delimited masses. A second type of metaplasmic inclusion following upon formation of the glycogen mass is found in the chromatoidal bodies. These lie adjacent to, or immediately on the periphery of, the glycogen mass, especially when the latter is large (pl. 31, figs. 9, 12) and in the earlier phases of their appearance. The nuclei are crowded to the periphery by the glycogen vacuole and may be somewhat flattened by its pressure (pl. 32, fig. 14). The chromatoidal bodies are often so numerous and so located as to obscure the nuclear structures beneath them. These bodies vary in number from one to twenty and are stout, rod-shaped, with rounded ends. They appear to be homogeneous and uniform in texture, though in some instances (pl. 31, fig. 11) the periphery is stained more deeply than the core. A similar disparity in staining of superficial and central portions is seen in an irregular siderophile mass closely encircling the nucleus observed by us in one cyst (pl. 31, fig. 10). The arrangement of these bodies gives no clear and consistent evidence that their location is determined by nuclear activity. There are often large rods (pl. 31, figs. 1, 7, 8, 11) or masses of rods in juxtaposition with the nucleus. However, they are often distributed over much of the periphery of the glycogen vacuole. In older cysts they are fewer, smaller, or entirely absent (pl. 32, fig. 20). The chronology of their formation and disappearance is indicative that their siderophile substance is in the metabolic cycle between glycogen and the living substance and that it has some relation to the process of growth as manifested in mitosis, and possibly to the anaerobic conditions of life within the cyst.

The Interphase

Plate 31, figure 1; plate 32, figures 19, 20

The interphase, or resting stage of the nucleus (pl. 31, fig. 3), in cysts is characterized by a considerable quantity of deeply stained peripheral chromatin applied rather uniformly throughout to the inner surface of the nuclear membrane. This distribution is prevalent in motile forms and cysts which have not been chilled. In cysts from stale stools and after chilling there is a tendency for the peripheral chromatin to be less evenly distributed and more or less deeply lobed in optical section, or gathered in discrete droplets on the membrane (pl. 31, fig. 1). It is probable that other conditions than chilling are at times associated with this blobbing of the peripheral chromatin, since in cysts from fresh stools on slides prepared in the usual routine, uneven distribution and more or less marked blobbing of the peripheral chromatin are not infrequently found.

The remaining deeply staining siderophile substance of the nucleus is found in the karyosome. This is a solid spheroidal body in a central or a slightly subcentral location. Its diameter is from 0.14 to 0.20 that of the nucleus and exceeds somewhat the thickness of the uniform layer of peripheral chromatin. An inspection of the location of the karyosome in many cysts demonstrates that often the karyosomes in the mononucleate and binucleate stages are not strictly central in location but are more or less off center. This is due to the approach of mitosis and is the first step in the migration of the karyosome to the periphery. In motile amoebas in the tissues in intestinal ulcers and elsewhere the karyosome is also found off center in some instances, presumably for the same reason. In the four-nucleate cysts the metabolic changes are less in evidence and a metabolic equilibrium is more nearly approached with the result that the karyosomes are more often centrally located.

A centriole or granule at the end of the paradesmose, distinct from and enclosed within the polar caps or daughter karyosomes such as we (Kofoid and Swezy, 1924) have found in *Karyamoeba* has not been differentiated by our methods, neither have we found such a granule in the central karyosome in the resting nucleus, such as Kühn (1920, pl. 11, figs. 3–5) finds in the karyosome of *Vahlkampfia bistadialis,* which divides in the prophase and at the metaphase appears as a granule in the center of each of the polar caps at the ends of an intradesmose.

Surrounding the karyosome is a narrow halo or clear zone whose width is often less than the diameter of the enclosed karyosome, but in some instances exceeds it (Kofoid and Swezy, 1924*a*, pl. 17, figs. 33–43). This halo is seen at the equatorial focal plane of the karyosome. In heavily decolorized nuclei it is difficult or impossible to demonstrate it. It even appears to be entirely absent in a few otherwise typical nuclei, indicating its relations to a considerable range of metabolic changes.

A third organelle of the chromatin complex, or siderophile substance of the nucleus, is the system of spoke radii which pass from the peripheral chromatin across the intermediate nuclear zone and the halo to the karyosome. They are slender filar structures, deeply staining, seemingly more numerous than the six chromosomes. There are suggestions, however, of their relationship to the emergence of the split chromosomes on the nuclear membrane. We are unable to demonstrate their number in the interphase, but the fact that the number is generally small at any optical section in which they can be traced, opens the possibility that the total is not far from twelve or that of the split chromosomes. At the prophase, however, they appear to be six in number, although often split distally.

THE PROPHASE

Plate 31, figures 2, 4–10

In this phase the karyosome migrates from its central position in the interphase to the periphery close to the nuclear membrane where it divides into two masses. These diverge and draw out the meridional intradesmose between them as they migrate to the poles of the forming spindle where they constitute the polar caps. The spoke radii are carried with the karyosome to the periphery and single or split threads radiating from the karyosome develop into the chromosomes by increase or transfer of siderophile substance. The spoke radii appear to be concerned in this process and may be the lines along which the chromatin material of the chromosomes is concentrated. We have insufficient data to determine the details of this process, especially the source or sources from which the chromosomes emerge. The facts that (1) during the splitting and thickening of the chromatin threads they seem to lie on or near the inner face of the nuclear membrane, and (2) that the peripheral chromatin grows less evident during this process, lead us to infer that the peripheral chromatin contributes mainly to their formation. It is very difficult to estimate the amount of chromatin in the transforming karyosome because of its changes in shape and its division, but the amount of chromatin it could contribute under seeming volumetric relations of the parts concerned cannot be very great. While the decrease in size of the karyosome is sufficient to provide some material for the formation of chromosomes, its substance primarily forms the polar caps. The emerging chromatin threads, forming presumably on the spoke radii, retain their connections with the two daughter karyosomes or polar caps by spindle fibers which often lie near the periphery in more or less meridional positions near the nuclear membrane. The diverging daughter karyosomes part the split chromosomes from their proximal end nearest to the karyosome to the distal end so that when they have attained their terminal position in the elongating spindle-shaped amphiaster as polar caps, the daughter chromosomes are in an end-to-end position astride or near the equator with their distal ends still joined or approximated and their proximal ones directed toward the polar caps.

The polar caps are thus in the morphological position of the centrosome. We have been unable in any mitotic phase to find evidence that the central karyosome contains within it a minute granule or centriole such as Jollos (1917) describes in *Vahlkampfia*, and as we found in *Karyamoeba falcata* (see Kofoid and Swezy, 1924*b*). The polar cap or centrosome in *E. dysenteriae* is much smaller than in *E. coli* (Swezy, 1922) or in *Councilmania lafleuri* (Kofoid and Swezy, 1921) and is itself little more than a granule. In a few instances the intradesmose appears to spread out as it merges into the proximal margin of the polar cap. In two instances, both in amoebas from the cat, we are able to find a polar granule rather sharply defined (pl. 33, figs. 22 and 27) at the ends of the intradesmose. One (fig. 22) is a late prophase with chromosomes in split pairs and the other is a telophase in which the chromosomes have fused in polar masses prior to constriction of the nuclear membrane. The thread crosses the chromatin masses and the centrioles at its ends lie in a halo-like area of the polar mass of chromatin.

The intradesmose is formed as the pairs of split chromosomes shorten up (pl. 33, fig. 22) and persists into the stage of nuclear constriction (pl. 33, figs. 25, 26). We are unable to trace it into the daughter nuclei. The intradesmose in completed form is a slender uniform thread, sometimes with a sigmoid curvature in its course.

THE METAPHASE

Plate 31, figure 11

This phase is designated thus by analogy with that of Metazoan mitosis. In it the nucleus is spindle-shaped with pointed ends in whose conical tips the polar caps are massed. The chromosomes are at the maximum of their condensation and distinctness and are grouped in the equatorial zone. There is not, however, a single equatorial plate of undivided chromosomes, but, owing to their precocious division in the prophase (pl. 33, fig. 22), the two sets of daughter chromosomes are in more or less of a pseudo-telosynaptic position. In some instances (pl. 31, fig. 11) they appear in part as single chromosomes, presumably fused end to end, while one or more pairs have parted and progressed in their movement toward their respective poles. At this phase the chromosomes are rods whose length is approximately twice their diameter. They are nearly uniform in size, though in most instances there is some evidence that one of the six is a trifle smaller than the other five. They do not divide synchronously so that the picture of their number may be confused in the early metaphase. For example, in one instance (pl. 31, fig. 11) there are eight chromosomes present, two daughter pairs and four undivided. In another spindle (pl. 31, fig. 12) there are eleven chromosomes, five daughter pairs and one undivided. The plane of divison in the metaphase is seemingly transverse to the elongated mass. This, however, is morphologically a longitudinal division since the chromosomes have previously split lengthwise in the prophase (pl. 31, figs. 9, 10) and lie in an end-to-end position in the metaphase as is the case in *Councilmania* (Kofoid and Swezy, 1921) and in *Trichonympha* (Kofoid and Swezy, 1919).

That the normal number of chromosomes is actually six and that the interpretations given are correct is proved by the number in the anaphase when the parting is completed. In this phase (pl. 32, figs. 13, 15) the number in each daughter group is clearly six.

The number of chromosomes is less clearly indicated in certain structural details, such as the number of chromatic threads radiating from the karyosome in the prophase when it is applied to the nuclear membrane (pl. 31, figs. 3–9). In some of these stages the distal ends appear to be bifurcated (fig. 10). This might represent either a splitting or refusion of previously split threads. We incline to the latter interpretation because of the indications (fig. 9) that (1) six pairs of closely parallel lines precede the contraction or condensation of the chromatin into six short chromosomes, and (2) the distal ends of these lines or chromosomes bifurcate or diverge. The six undivided chromosomes emerging in the early prophase often show distal bifurcations (fig. 10). Unfortunately we are yet unable to find morphological evidences which reveal fully the shift from the parallel to the end-to-end relation of the daughter chromosomes. The supposedly split pair of daughter chromosomes appears to behave as a single plastic unit and later to constrict transversely in the middle at the seeming end-to-end synapse.

The intradesmose is at its climax in this stage. In the prophase as the two daughter karyosomes (pl. 31, figs. 9, 10) move apart on the inner face of the nuclear membrane, they draw out of their own substance a slender siderophile thread which, upon their arrival at their final polar location, lies in a meridional position as a slender thread of uniform caliber joining the polar caps or centrosomes at the poles (pl. 31, figs. 11, 12). This thread is most clearly seen when it is located on the upper or lower surface of the nucleus. In a lateral position it may easily be overlooked on the nuclear membrane. It is usually not strictly meridional but has a slight sigmoid curvature. It is always heavier than the spindle fibers which also lie on or near the nuclear membrane and is detected with difficulty by slow change of focus.

THE ANAPHASE

Plate 31, figure 12; plate 32, figures 13–17

This phase impinges upon and overlaps the preceding one. It is characterized by the divergence of the daughter chromosomes and their arrival at the poles of the spindle-shaped nucleus. Since the chromosomes do not part and diverge synchronously the metaphase and anaphase are to this extent interdigitated.

This phase in mitosis is characterized in our material both in cysts and in motile amoebas from man by the movement of the chromosomes without noticeable modification to the poles, where, for a time at least, they cluster and tend to fuse (pl. 33, figs. 24–26). In this position their number may be obscured, but is indicated by their projecting ends.

THE TELOPHASE

Plate 33, figures 25–27

In this phase the nucleus constricts, the chromosomes string out along the meridional spindle fibers, and the peripheral chromatin and karyosome reappear. It is quite probable that under certain conditions the disintegration of the chromosomes may be accelerated. It is conceivable that such disintegration may be more or less pathological or abnormal, as in the height of acute dysentery of kittens in which the protein derivatives of the cells of the host are at a climax of their production.

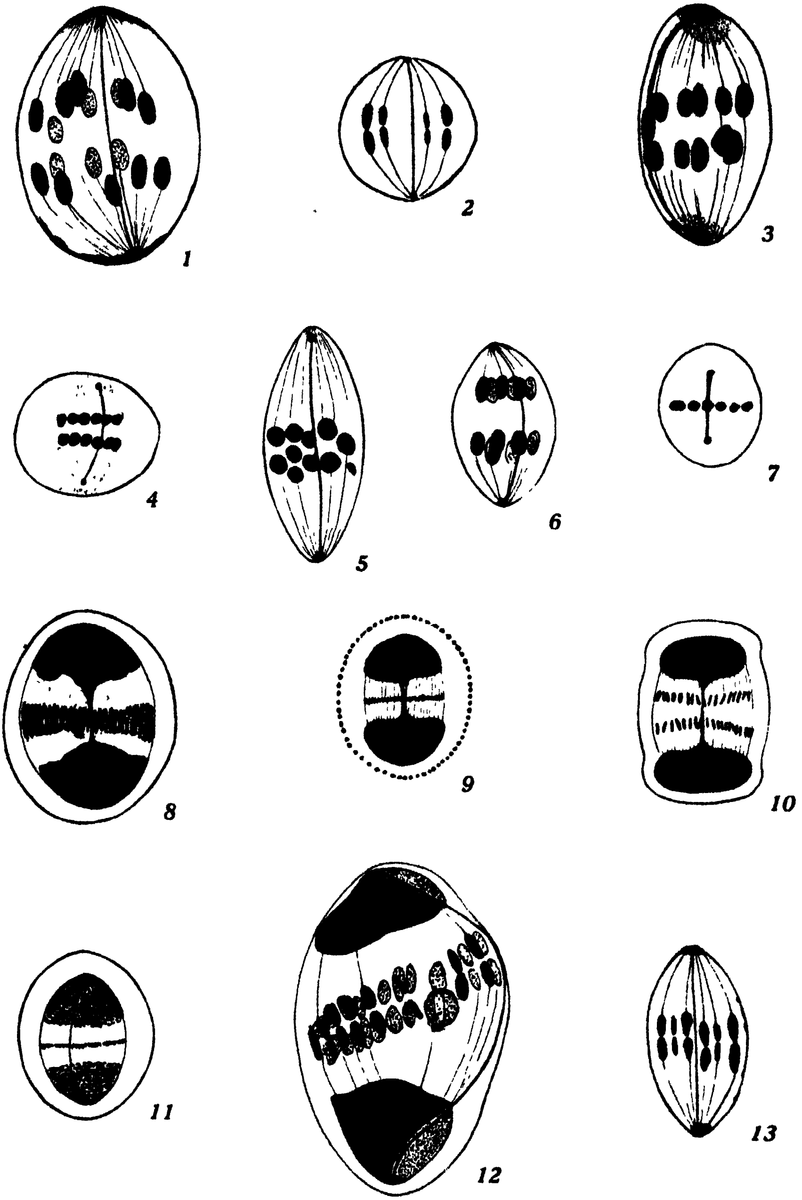

Fig. A. Nuclei at or near the metaphase of mitosis in various genera and species of the Amoebina. × 4000. Figures A 1–3, 5, 6, from cysts, the others from motile amoebas.

1. Nucleus of *Councilmania lafleuri* Kofoid and Swezy. Original from binucleate cyst.

2. Nucleus of *C. decumani* (Rudovsky) Kessel. From a four-nucleate cyst. After Kessel (1924, pl. 47, fig. 45).

3. Nucleus of *Endamoeba coli* (Loesch) from a binucleate cyst. After Swezy (1922, pl. 29, fig. 9).

(Description continued on p. 341.)

It is evident that in the type of telophase in the cysts from stools and in active amoebas from the margins of intestinal ulcers in man the chromosomes at the beginning of the telophase lie in clusters at the poles. Their dispersal in the peripheral chromatin seems to follow (pl. 33, fig. 26) rather than precede the initiation of nuclear constriction.

Our grounds for concluding that this is a normal rather than an abnormal process are that it occurs both in cysts and in motile amoebas although the latter are in some instances only from necropsy material.

DISCUSSION

As previously stated most of Dobell's figures represent anaphases and telophases and are drawn from motile amoebas in experimental infections in the cat. This animal is not the normal host. Encystment is not completed in it and the invasion and destruction of the living tissues of the colon is rapid and extreme. How far this results in modifications of and disturbances in the sequences in mitotic changes cannot at present be stated. It is perhaps sufficient in the premises to regard mitosis as found in motile amoebas in tissues and in cysts *in man* as the normal process. Dobell's (1919) figures at the best represent only a part of the process in a non-normal though partially adapted host.

The type of mitosis we have previously found in *Councilmania* (fig. A, 1) and in *Endamoeba coli* (fig. A, 3) is essentially the same as that we now describe in *E. dysenteriae*. It conforms also in the main to that described by Mathis and Mercier for *E. dysenteriae* (1916) and also for *E. legeri* from *Macacus* (Mathis and Mercier, 1917; our fig. A, 5).

4. *Hartmannella aquarum* Jollos. From a mononucleate amoeba. After Jollos (1917, pl. 16, fig. 70).

5. *Endamoeba legeri* Mathis. From a binucleate cyst. After Mathis and Mercier (1917, fig. 11, d).

6. *Endamoeba dysenteriae* (Councilman and Lafleur) Craig. Original from a mononucleate cyst.

7. *Amoeba hyalina* Dangeard. After Hartmann and Chagas (1910, pl. 10, fig. 12).

8. *Amoeba diplogena* Bělař. After Bělař (1915, pl. 2, fig. 26).

9. *Vahlkampfia limax* (Dujardin). After Vahlkampf (1905, pl. 6, fig. 5).

10. *Vahlkampfia diplomitotica* Aragão. After Aragão (1909, pl. 2, fig. 18).

11. *Vahlkampfia lacertae* Hartmann. After Jollos (1917, pl. 15, fig. 52).

12. *Karyamoeba falcata* Kofoid and Swezy. After Kofoid and Swezy (1924, pl. 24, fig. 14).

13. *Councilmania muris* (Grassi). After Kessel (1924, pl. 44, fig. 17).

To conclude that their account is "incomplete and faulty" (Dobell, 1919, p. 43) and to reject our accounts of the process as Wenyon and Manson-Bahr have done in their reviews of our work, is apparently to conclude that Dobell's account alone is complete and accurate. It is our judgment in the light of our material and its analysis that his stages are inadequate in range and number and his analysis is so incomplete as to require further elucidation and emendation.

With a view to facilitating a comparison of our findings on the type of mitosis in *Endamoeba dysenteriae* with those of other protozoologists on various types of the Amoebina we have assembled in figure A reproductions from various sources. We have chosen for this comparison the critical stage of the amphiaster or metaphase in which the polar caps or centrosomes, the intradesmose, and the chromosomes are most clearly and definitely demonstrable. In figures A, 1–3, 6, and 13 are shown metaphases of *C. lafleuri*, *C. decumani*, *E. coli*, *E. dysenteriae*, and *C. muris* as determined by us or by Dr. Kessel, in which the nuclear structures above enumerated are present in comparable condition, showing morphological relations and cytological structure of a common pattern. The characteristics are polar centrosomes, a meridional intradesmose, a definite and clearly organized group of chromosomes in a double equatorial plate, and an intact nuclear membrane. *E. legeri* and *Hartmannella aquarum* are unquestionably of a closely similar type. *Amoeba hyalina* conforms to this type in essential characters, the stage figured being a face view of what seems to be an early stage in which the centrosomes have not reached the poles and the chromosomes have not yet parted.

The *Vahlkampfia* group (our fig. A, 8–12) includes one metaphase drawn by us (Kofoid and Swezy, 1924*b*) of *Karyamoeba falcata*, a genus which clearly belongs in the *Vahlkampfia* section of the Amoebina. This section has a metaphase in which the nuclear figure is more or less separated from the nuclear membrane by a clear zone, has very large and massive polar caps, and what is probably in all cases a meridional intradesmose, though this is figured by others as located in a position suggestive of an axial location; but this position is probably a face view instead of a lateral one. The chromosomes in all instances are numerous and are very small in *Vahlkampfia*, though not in *Karyamoeba*. It seems that the latter genus alone has the persistent spherical karyosome-like body (fig. A, 12). This section likewise exhibits the characteristic features of persistent nuclear membrane, intradesmose, polar caps, and, at least in some instances if not in all, definite chromosomes assembled in an equatorial plate.

Our findings as to the type of mitosis in the nuclei of human intestinal Protozoa in both encysted phases in the stools and in the motile phases in the intestinal ulcers, bone marrow, and lymph glands are thus in consonance with those of other investigators of mitosis in the Amoebina who have attempted to describe the process and have succeeded in finding the essential stages.

It is difficult for us to conceive that the cells we have interpreted as amoebas in bone marrow and lymph glands should exhibit nuclei both in the interphases and in mitosis conforming so exactly, so minutely, and so consistently to the morphological type of nucleus and mitosis in the Amoebina and at the same time be merely abnormal human cells. Furthermore, the constancy in chromosome number throughout the process is highly significant. The structural similarity is too striking and too uniformly consistent to conform to the well-known abnormalities of aberrant human cells. In fact such cells are noticeably absent from the preparations in which the amoebas occur.

To those who work with these minute structures no explanation of the difficulties is needed. To others it may be of interest to note that the process of mitosis is carried out in nuclei from five to eight microns in diameter or length of spindle and that the chromosomes are often less than a micron in length, while the intradesmose and spindle fibers are at the very limits of magnification and definition with the best obtainable optical equipment.

An adjunct of great assistance in the use of the monobjective binocular is an eye shield with three sides about six inches in height attached above the prism box of the tube and fitted around the bases of the tubes for the oculars. This shield is finished in opaque black and relieves the observer from the interference due to lateral light. An adjustable rheostat on a high grade microscope lamp also assists in maintaining uniformity of illumination and in modifying it as desired.

We have not noted one feature of the mitotic process, namely, the fact that the nuclear membrane persists throughout mitosis. It is quite devoid of a well-defined peripheral chromatin layer, at least in thoroughly decolorized nuclei, and is therefore sometimes difficult to trace except in direct, optical transsection. Its persistence during this period appears to be a constant characteristic of the Amoebina, and probably also of the Mastigophora, while in the Metazoa generally the nuclear membrane entirely disappears at mitosis, is reformed about the clustered chromosomes at the close of the anaphase, and constitutes the most striking feature of the telophase. The redistribution of the chromatin from the polar cluster at the close of the anaphase in *E. dysenteriae*, so that it again forms a peripheral layer throughout the inner face of the nucleus is comparable with the reformation of the membrane in the Metazoa and takes place at a comparable stage in the mitotic process.

It is probable, as shown in the figures of Jollos (1917) and in the anaphases and telophases of Dobell's (1919) plate, that the chromosomes redistribute themselves in blobs along the lines of the meridional spindle fibers on or near the nuclear membrane and thus achieve a symmetrical distribution. In passing, it may be noted that these important parts of the mechanism of heredity are thus placed in a position of maximum opportunity for contacts with such interchanges of substances between nucleus and cytoplasm as metabolism may involve.

Reference is made here to the fact that the *persistence* of the nuclear membrane is characteristic of mitosis in the *amoebas* in contrast with its *disappearance* in the Metazoa, since Manson-Bahr (1922, p. 384) in his review makes the following statement:

> The disappearance of the nuclear membrane during mitosis and the formation between the daughter centrosomes, at the poles of the spindle, of a type of connexion known as the central spindle, or centrodesmose, are quoted as features which distinguish these 'amebas' from surrounding cells.

This statement is unfortunate since the characteristics he names are just those which characterize mitosis in Metazöan cells and not mitosis in the Amoebina. His statement is, moreover, directly the reverse of the statement made in the paper he reviewed (Kofoid and Swezy, 1922*a*) and might be misleading to his readers.

SUMMARY

The process of mitosis in motile and in encysted stages in *Endamoeba dysenteriae* from human stools and human tissues has been found to be consistently of one type, namely, that in other Amoebina. The prophase is indicated by the migration of the central karyosome to the inner face of the nuclear membrane, taking with it the filar spoke radii. In the early stages of this process there are indications that the spoke radii are split lengthwise. The karyosome divides and the daughter karyosomes spin out a slender intradesmose between them as they move into polar positions on the elongating nucleus where they form the polar caps or centrosomes. The intradesmose is a slender uniform thread on the nuclear membrane in a meridional position and often has a sigmoid curvature. It persists till the early telophase. The chromosomes are six in number and emerge on the spoke radii. They appear first as single masses and later as split pairs in a side-by-side position. This is followed by an end-to-end position on the equatorial plate of the metaphase and migration to the poles in the anaphase. The nuclear membrane persists throughout the process. The daughter nuclei separate by constriction. The process is a normal morphological one and is the same in the cysts and in motile amoebas. It is not to be confused with the cytological behavior of abnormal human cells.

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EXPLANATION OF PLATES

All figures are camera lucida drawings of *Endamoeba dysenteriae* (Councilman and Lafleur) Craig from smears of human stools, except as stated, fixed with hot Schaudinn's fluid and stained with iron haematoxylin. × 2500.

PLATE 31

Fig. 1. Mononucleate cyst with small glycogen vacuole, sausage-shaped chromatoidal body, and nucleus in the interphase with blobbed peripheral chromatin, central karyosome, halo, and spoke radii.

Fig. 2. Early prophase showing beginning of migration of karyosome. Glycogen toward the periphery. Spoke radii show accumulating chromatin, vacuoles diffused, chromatoidal small.

Fig. 3. Mononucleate cyst with uniformly distributed peripheral chromatin, small halo, much diffused cloud of glycogen vacuoles, vaguely stained chromatoidal (?) mass.

Fig. 4. Early prophase showing excentric karyosome and chromatin collecting in granules along spoke radii. Small amount of peripheral chromatin.

Fig. 5. Early prophase with peripheral karyosome, very large glycogen vacuole, and eight stout chromatoidals with central portion more lightly stained.

Fig. 6. Early prophase with excentric karyosome and glycogen vacuole broken up in numerous small vacuoles. Chromatoidal substance represented by a single slender rod.

Fig. 7. Early prophase with peripheral, lobed, ring-shaped karyosome and two very stout chromatoidals. Small amount of peripheral chromatin.

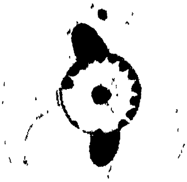
Fig. 8. Early prophase with chromatin of karyosome drawn out on the spoke radii. Diffuse glycogen vacuoles and two typical chromatoidal rods.

Fig. 9. Early prophase with karyosome-centrosome divided, the moieties drawing out the intradesmose between them as they move apart. Spoke radii enlarged, and showing some evidence of splitting. Small amount of peripheral chromatin. Hugo glycogen vacuole with chromatoidals massed about it.

Fig. 10. Centrosomes with connecting intradesmose and six chromatin granules (chromosomes) on linin fibrils split distally in some cases. Chromatoidal substance massed around nucleus, with lighter central region. Single glycogen vacuole crowds the nucleus and chromatoidal mass to one side.

Fig. 11. Metaphase with two chromosomes separating but others not yet parted in transverse plane. Intradesmose connecting centrosomes at poles of spindle inside the nuclear membrane. Chromatoidals massed near nucleus and surrounded by the glycogen vacuoles.

Fig. 12. Daughter chromosomes moving towards poles with a lagging one still not parted. Numerous stout rod-like typical chromatoidals around the very large glycogen vacuole which crowds them and the nucleus peripherally. Sigmoid intradesmose on the inner face of the nuclear membrane.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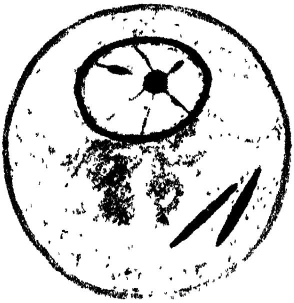
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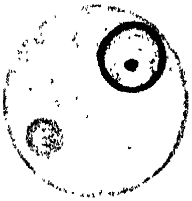
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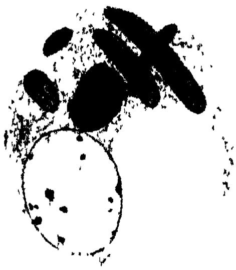
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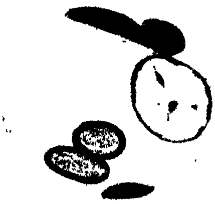
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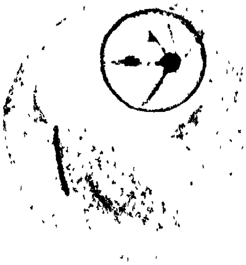
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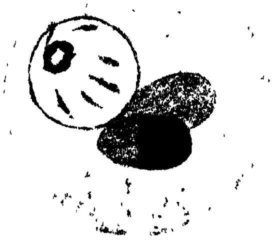
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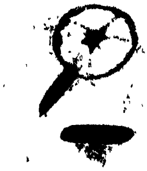
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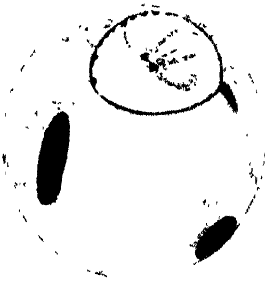
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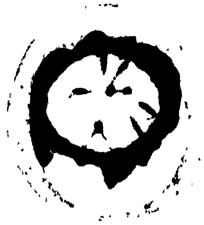
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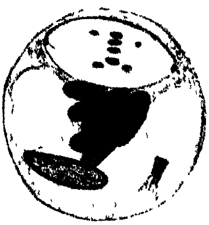
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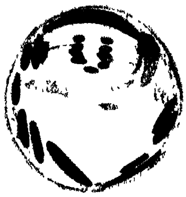
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PLATE 32

Mitosis in cysts of *Endamoeba dysenteriae* (Councilman and Lafleur) Craig. × 2500.

Fig. 13. Binucleate cyst with nuclei in early anaphase. Chromosomes shown only in upper nucleus with one pair lagging. Small glycogen vacuole with a siderophile sphere in its margin.

Fig. 14. Late anaphase in a mononucleate cyst with two chromosomes still not parted. Many chromatoidals on the border of the very large glycogen vacuole.

Fig. 15. Anaphase in a mononucleate cyst with chromosomes moving synchronously toward the poles. Chromatoidals massed in the center with several glycogen vacuoles about them.

Fig. 16. Polar view of a spindle in the anaphase in a mononucleate cyst. Intradesmose at the left. Nucleus flattened against the large glycogen vacuole. Stout chromatoidals crowded adjacent to nucleus and vacuole.

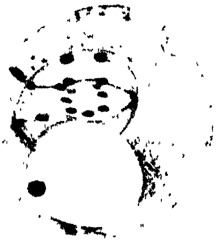
Fig. 17. Similar anaphase viewed from end with six chromosomes attached to each centrosome by fibers. Intradesmose is to be seen as a loop on the left.

Fig. 18. Binucleate cyst in the interphase.

Fig. 19. Binucleate cyst with single, typical, sausage-shaped chromatoidal and one of long, thread-like type.

Fig. 20. Cyst with four nuclei showing uniformly distributed peripheral chromatin and karyosomes centrally located.

Fig. 21. Active amoeba from stool with abnormal type of mitotic figure. Centrosomes and intradesmose normal in appearance, but chromosomes massed and indistinct.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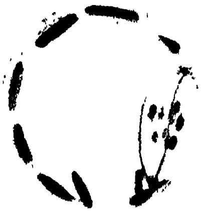
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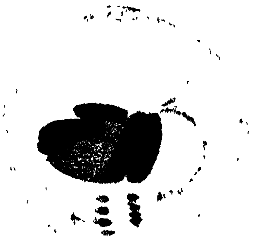
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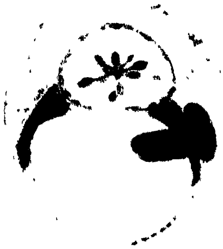
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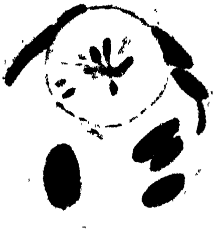
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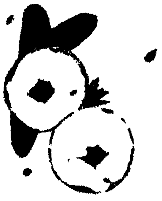
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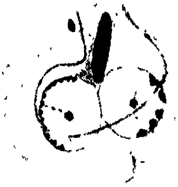
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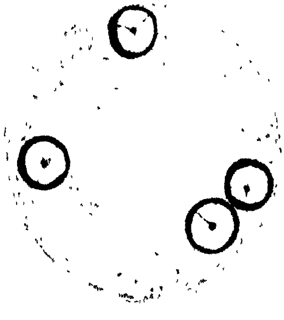
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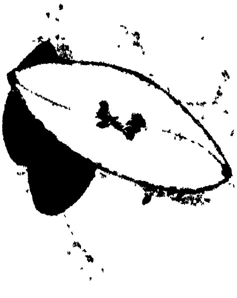
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PLATE 33

All figures of active phases of *Endamoeba dysenteriae* (Councilman and Lafleur) Craig. × 2500.

Fig. 22. Late prophase in an active amoeba from a section of an amoebic ulcer in a human colon. Nucleus shows centrosomes connected by a sigmoid intradesmose and six paired chromosomes in a side-to-side position, prior to their arrangement in an end-to-end double equatorial plate.

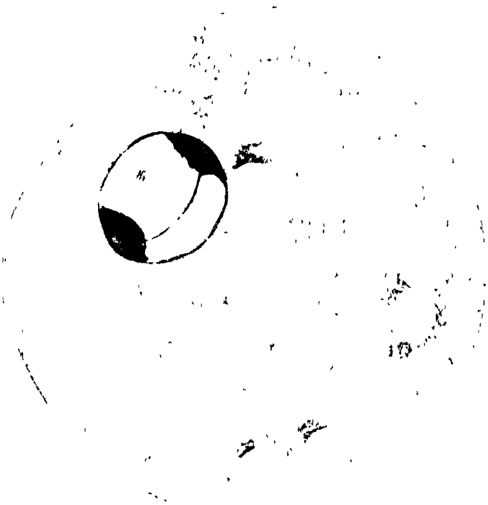
Fig. 23. Active amoeba from a smear after autopsy from an ulcerating colon of cat infected with *E. dysenteriae* from an acute human case. An early telophase with the chromosomes massed at the poles and spreading out. Intradesmose very distinct, but centrosomes obscured (see fig. 27). This nucleus has just begun to elongate but is far advanced in other mitotic features.

Fig. 24. Active amoeba from same section as figure 22. Nucleus with chromosomes with their proximal ends still protuberant, grouped at the poles which are still connected by the peripherally located intradesmose. Polar centrosomes merged in the chromosomal mass.

Fig. 25. Telophase in an active amoeba from same section showing nucleus with nuclear membrane beginning to constrict to form the two daughter nuclei. Intradesmose only faintly stained.

Fig. 26. Later telophase in an active amoeba from a smear after autopsy from the ulcerating colon of an experimentally infected cat. Nucleus elongating with membrane beginning to constrict and the intradesmose fading out.

Fig. 27. Telophase of an active amoeba in the same smear as the amoeba of figure 26. The intradesmose is very dark near the poles and faint in the intermediate area. It terminates in a distinct granule in a clear halo at the slightly conical pole. Immediately around it is the massed chromatin material derived from the fused chromosomes which is spreading over the nuclear membrane.



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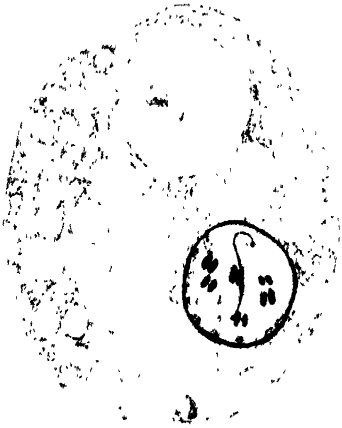

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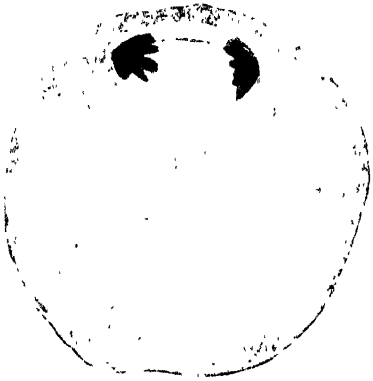

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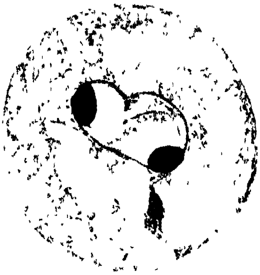

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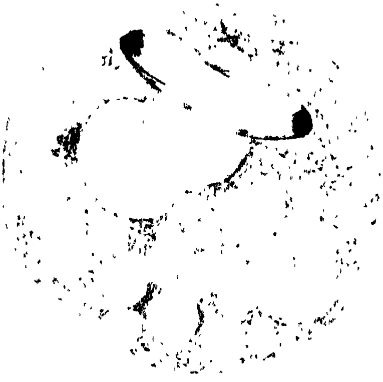

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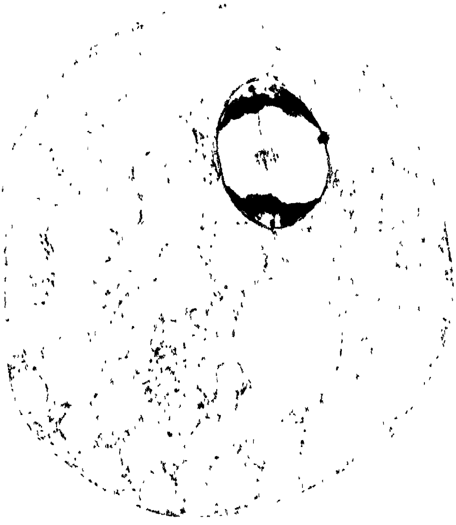

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STUDIES ON PARAMECIUM

I. EXPERIMENTS ON THE ACTION OF VARIOUS ENDOCRINE SUBSTANCES, OF LIVER, AND OF GLYCOGEN, ON THE DIVISION RATE OF PARAMECIUM

BY

GORDON H. BALL

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INTRODUCTION

The work of Jollos (1921) indicates the possibility of inducing hereditary changes in *Paramecium* by means of external agents. The use of solutions of endocrine glands, particularly thyroid glands, seems to offer a favorable means of attacking the problem. The marked effect of thyroid on the metabolic rate of Mammalia and of Amphibia has been firmly established; and the work of Nowikoff (1908), Budington and Harvey (1915), and Shumway (1914 and

1917) showed that thyroid produces a decided increase in the division rate of *Paramecium*. Further, if it is possible to influence the germinal material by external factors, probably one of the principal means of stimulation is by the chemical route (Guyer and Smith, 1920). Under such circumstances, the rôle of hormones in effecting germinal changes might be considerable.

After a few preliminary experiments, an attempt was made, beginning in June, 1922, to determine the effects of desiccated thyroid on the division rate of *Paramecium*. Although some of the tests showed a decided increase in the rate of multiplication for the thyroid-fed lines, other results were contradictory. This led to a more intensive investigation of the effects of feeding desiccated thyroid to *Paramecium*, under carefully controlled environmental conditions. The results of this study form the substance of the present paper.[1]

ACKNOWLEDGMENTS

The experiments were carried on under the direction of Dr. Charles A. Kofoid, whom the writer wishes to thank for his invaluable suggestions and criticisms. Thanks are also due Dr. H. M. Evans and Dr. P. E. Smith of the Department of Anatomy of the University of California, for opportunity to obtain fresh bovine hypophysis. The completion of the work was made possible for the writer by the occupancy of the University Fellowship in Zoology for 1923–1924.

HISTORICAL REVIEW

The effect of various physical and chemical agents on the division rate of ciliates, especially of *Paramecium*, has been the subject of many experiments. Since many of these do not bear directly on these investigations, they need not be treated at length.

PHYSICAL STIMULI

Division appears to proceed at an equal rate in light and in darkness (Maupas, 1888; Woodruff, 1905); although Dembowski (1922) recently claims to have found a noticeable decrease in the division rate of *Paramecium* kept in the dark, as compared with those kept in the light. Blue was unfavorable for multiplication; ultra-violet light was even less suitable. These results are open to question, however, as there seems to have been no attempt to insure approximately equal numbers of bacteria in the different experiments; and blue and ultra-violet rays are known to be strongly germicidal. On the other hand, Forbes and Daland (1923) and Bovie and Daland (1923) find a direct harmful effect of ultra-violet light on *Paramecium,* indicated by a lowered heat resistance.

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[1] The recent paper of Woodruff and Swingle (1924) appeared just as the present work was going to press. Although the work was done independently, the two studies cover much of the same ground and come to the same general conclusions.

X-rays appear to be harmful to *Paramecium* (Baldwin, 1920). Although radio-active substances are destructive if allowed to act too long (Veneziani, 1904; Zuelzer, 1905; Packard, 1924), yet a brief exposure seems to produce a temporary increase in the rate of division (Markovits, 1922; Packard, 1924).

The effects of mechanical stimuli on division rate are variable, and the results may be due possibly to other factors. Calkins (1902) found that mechanical shaking increased the division rate during a depression period, but decreased it during a period of rapid multiplication. In later depression periods, shaking was without effect; and Morse (1910) found no change in the division rate of *Paramecium* which had been mechanically agitated. Centrifuging does not appear to alter the division rate; McClendon's figures (1909), which show an increase, are subject to a possible error in that he compared his centrifuged animals with controls which died off or had an abnormally low division rate, a criticism previously made by Dobell (1914). Crampton (1912) found that *Paramecium* could be kept for many days in capillary tubes without dividing, although, at intervals, they appeared to throw off pieces of cytoplasm.

It has long been known that the rate of division increases, within certain limits, as the temperature rises. Spallanzani (1776) was apparently the first to observe that ciliates multiplied faster at high than at low temperatures. This has been confirmed by a number of observers, of whom we mention only Maupas (1888), Bütschli (1887-1889), Joukowsky (1898), Woodruff (1905), Popoff (1908), Woodruff and Baitsell (1911), Sun (1912), and Middleton (1918). In general, the acceleration in division rate suggests a relation to Van't Hoff's law. In the flagellate, *Chilomonas paramecium,* Maltaux and Massart (1906) found that a sudden rise in temperature produced a large number of dividing forms within a few minutes. No such acceleration of division rate seems to occur in the ciliates when the temperature is suddenly raised.

EFFECTS OF CHEMICALS

The failure of *Paramecium* to divide in distilled water is due to the lack of food; pure distilled water, in itself, is not toxic (Daniel, 1908; Estabrook, 1910). The rôle of inorganic salts in altering the division rate of ciliates has been studied recently by Spek (1919, 1920), who found that those salts which tend toward the solation of the cellular colloids or toward the increase of the water content of the cell increase the division rate. He showed that LiBr, LiCl, and KSCN, were particularly favorable. Although Spek states that he eliminated any effect due to the number of bacteria present, yet his results may not be statistically significant, since his tables give numbers of animals rather than numbers of divisions. Zweibaum (1912) mentions a large number of salts which are favorable for the multiplication of *Paramecium*, but gives no figures comparing the salts with one another or with the controls.

Unfortunately, there seems to be no consensus of opinion as to the effect of inorganic substances on the division rate of ciliates, probably because of the fact that different workers experiment on races with different physiological properties. The occurrence of clones of this type in a population of *Paramecium* was first pointed out by Jennings (1908*b*); and Spek mentions (1920) that several of the races with which he worked consistently failed to show any positive effect with salts favorable for the other lines. References to the effects of different inorganic salts on division rate may be found in Calkins (1904), Calkins and Lieb (1902), Estabrook (1910), Middleton (1922), Peters (1904), Sand (1901), Spek (1919), Sun (1912), Woodruff (1905), and Zweibaum (1912).

Contradictory results have also been found for many of the organic compounds. Calkins and Lieb (1902) found that alcohol increased the division rate of *Paramecium*. This is contrary to the results of Sand (1901), which indicated a decreased division rate for lines treated with alcohol. Woodruff (1908) showed that the effect of alcohol on division rate varied with the period in the life-cycle at which it was applied; while Matheny (1910) obtained either no change at all or a lowered rate of division. Maltaux and Massart (1906) found that alcohol acted similarly to a sudden rise in temperature in accelerating division in *Chilomonas*.

Behrend (1916) showed that a low concentration of glycerine increased the fission rate of *Colpidium;* Peters (1904) demonstrated an increased rate in *Paramecium* following the addition of small amounts of chloroform; Browder (1915) found a heightened division rate with cholesterol and a decrease with lecithin. Quinine lowers the division rate according to Giemsa and Prowazek (1908) and Acton (1921), but Sand (1901) finds it increased with appropriate concentrations. Strychnine produces a temporary increase according to Calkins and Lieb (1902) ; Sun (1912) finds the rate decreased with uric acid.

EFFECTS OF METABOLIC PRODUCTS

Extracts from nephritic kidneys lower the division rate while those from normal kidneys are without effect (Woodruff and Underhill, 1913). Carcinomatous tissue increases the division rate in low dilution, and decreases it in high concentration (Underhill and Woodruff, 1913). The later products of nucleo-proteid hydrolysis increase the division rate of *Actinobolus* (Calkins, Bullock, and Rohdenburg, 1912) ; although the excretion products of *Paramecium,* itself, appear to be toxic and to decrease the rate of fission (Sun, 1912; Woodruff, 1911, 1913). Robertson (1923) states that the bacteria of a culture secrete a substance into the medium, which, when acted upon by Infusoria, accelerates the rate of division. According to him, the division rate decreases as the volume of the fluid available increases; and two animals of the same strain and age exert a mutual autocatalytic effect on the rate of fission. These experiments are opposed to Woodruff's findings (1911), that the division rate varies directly with the volume of fluid; and Greenleaf (1924) has been unable to confirm Robertson's results. Animals from old infusions divide less rapidly when transferred to fresh media than do those from young infusions (Joukowsky, 1898; Robertson, 1923; Cutler and Crump, 1923).

EFFECTS OF BACTERIA

The effect of the bacteria of the culture media and of different strains of bacteria on division rate has not received the attention which it merits. Hargitt and Fray (1917) found that mixed cultures of hay-infusion bacteria were preferable to single strains; and that the division rate varied considerably depending upon the kind of bacteria used. Their work has been abundantly confirmed by the results of Phillips (1922) and of Chatton and Chatton (1923).

EFFECTS OF VITAMINS

According to Calkins and Eddy (1917), pancreatic vitamin does not alter the division rate; Flather (1919*a*) found polished rice less favorable as a food than unpolished rice, but neither gave so high a division rate as did the control, fed on malted milk. Orange juice did not alter the rate of fission. Chambers (1919) found no increase with solutions of potato; while yeast increased the division rate under some conditions but was ineffectual in others. In all of these experiments on vitamins, there seems to have been no attempt to insure approximately equal numbers of bacteria in the control and in the experimental lines.

EFFECTS OF GLANDULAR SUBSTANCES

Nowikoff (1908) was apparently the first to investigate the effect of thyroid on the division rate of Protozoa. He found that desiccated sheep's thyroid, dissolved in distilled water, produced a large increase in numbers of *Paramecium caudatum* as compared with hay infusion. For several reasons, however, his results are open to question. He did not isolate the ciliates daily, but merely counted them each day until the culture died out. Consequently, he did not eliminate the possibility that he was measuring merely the relative values of the two substances as bacterial food; or that a secondary influence due to the accumulation of excretory products might be the explanation of his results. Again, according to his figures, the total number of divisions is approximately the same with thyroid and with muscle as the source of food. And finally, as Shumway has shown (1914), when the total number of divisions instead of the total number of organisms is considered, the increase in rate is practically eliminated.

Budington and Harvey (1915) tested thyroid-fed *Paramecium* (sp.?) and *Stylonychia* against sister organisms fed on hay infusion, and found a higher division rate in the thyroid-fed group. They also counted the number of organisms rather than the number of divisions, and did not isolate daily. They found an increased division rate with freshly desiccated glands from each of the five classes of vertebrates.

Shumway (1914, 1917) was the first to employ the method of daily isolation used by Calkins, Woodruff, and others. He worked with both fresh and desiccated glands, and with both *Paramecium aurelia* and *P. caudatum.* He found an average increase in the division rate in each species of approximately 65 per cent over that of the control on hay infusion. The thyroid-fed individuals were smaller, more active, and more transparent, with vacuolated protoplasm and a tendency toward the formation of three contractile vacuoles—all of these findings pointed to an increased metabolic rate.

Abderhalden and Schiffmann (1922) obtained similar results with *Paramecium,* using the thyroid "optone"; they did not isolate the animals daily, however. They also noted that the higher division rate was not manifest until about the fourth day of the experiment.

Cori (1923), isolating daily, found an increase of 12 per cent with *P. putrinum*, fed with desiccated thyroid, as compared with the control on hay infusion.

Riddle and Torrey (1923), using thyroxin, were unable to obtain an increased division rate of *Paramecium;* and Torrey (1924) recently found a pronounced decrease with the thyroxin-fed lines. Thyroxin appeared to produce a marked heightening of the excretory processes.

Woodruff and Swingle (1923, 1924), using a pedigreed race of *Paramecium aurelia*, and isolating daily, found that thyroxin either lowered the division rate or produced no effect, as compared with the control, which was fed on beef extract. Thyroxin did not increase the division rate of *Paramecium* living on media to which fresh frog-pituitary had been added; and, although thyroid increased the division rate, the animals divided as rapidly in media prepared from freshly desiccated muscle as in that made from freshly desiccated thyroid. These authors point out the possibility that earlier positive results with thyroid were due to variations in the bacterial food supply.

A few experiments have been carried out with respect to the effects of other kinds of glands on division rate. Desiccated hypophysis causes no significant increase in the rate of division (Nowikoff, 1908; Shumway, 1917); a slight increase (Chambers, 1919); a decrease followed by a marked increase upon transfer to a fresh solution (Abderhalden and Schiffmann, 1922, using hypophysis "optone"); either an increase or no effect (Woodruff and Swingle, 1924); an increased contractile vacuole rate (Flather, 1919*b*). Woodruff and Swingle find a heightened division rate with fresh frog-pituitary over that of the control with beef extract. Desiccated suprarenal does not increase the division rate according to Nowikoff and Shumway; produces a slight increase (Chambers); while adrenalin accelerates the contractile vacuole rate (Flather). Tethelin, according to Robertson

(1923), increases the rate of division to a considerable extent. Shumway finds no significant increase with extracts of desiccated thymus, pancreas, spleen, or ovary, although only the experiments with thyroid and thymus are sufficiently extensive to warrant any general conclusion. Abderhalden and Schiffmann, on the other hand, find as marked an increase with thymus as with thyroid; and an increase with testis optone but a decrease with corpus luteum. According to Flather, pineal extract causes a more rapid contraction of the pulsating vacuole, while Woodruff and Swingle find that desiccated pineal sometimes accelerates the division rate, but at other times has no effect. The latter workers state that their positive results with pineal and pituitary are similar to those they obtained with thyroid, and come about merely because of better food conditions and not because of any specific hormone secreted by the glands.

MATERIAL AND METHODS

The experiments which form the subject of this paper cover a period of approximately two years. With perhaps half a dozen exceptions, the organisms were counted and isolated daily during the course of each experiment; in no case were they carried past two days without transfer to fresh food. Each day, the number of *Paramecium* in the culture was counted, and a single animal transferred to a new depression slide to which fresh food had been added. Four drops of fresh media were taken as a constant volume in every transfer. The slides were stacked in moist chambers, which consisted of stender dishes averaging 150 mm. in diameter and 70 mm. in height. At the bottom of the stender was placed a glass disk with raised parallel bars, such as is found in Moore's staining dishes. By stacking the slides so that they form a square, it is possible to place from thirty-five to forty-five slides in one stender dish. It is useful to have the surface of the slide, except the depression, made of frosted glass, since this prevents the water from spreading and permits rapid marking of the slide with pencil. This mark can be removed only after more or less vigorous rubbing; consequently there is no danger of destroying the record. Both the control and the experimental lines for any one race were carried in the same moist chamber, and all the chambers were placed next to one another on the same table. In this way, any effect from temperature differences was eliminated. All the dishes were kept at room temperature.

The organisms were transferred by means of a capillary pipette, which was rinsed by filling with boiling water before each isolation. They were counted under a binocular dissecting microscope, carrying a 55 mm. objective and No. 2 oculars, giving a magnification of 16.

Distilled water was used for making all the solutions. The hay infusion was prepared by boiling ten grams of timothy hay in one liter of distilled water for ten minutes. When it was desired to keep the solution sterile for any length of time, it was autoclaved at twenty pounds pressure. The thyroid was Armour's desiccated preparation, iodin 0.2 per cent. Thyroid purchased on two different occasions was used, and both times gave similar results. The strength of the thyroid solution was 1 mg. to 10 cc. hay infusion, a greater amount than dissolves at room temperature. The glycogen powder was prepared by Merck and by Pfanstiehl (C. P.). In the experiments with hypophysis, fresh beef hypophysis was obtained daily from the Department of Anatomy, the anterior and posterior lobes separated, and a portion of each thoroughly ground up in a mortar with sand and distilled water. This was then centrifuged, and the supernatant liquid used. Liver from freshly killed rats was ground up and centrifuged in the same way.

In each experiment, the animals used belonged to the same clone. These clones, each originally derived from a single individual, have been carried in quarter-pint milk bottles plugged with cotton stoppers and have been transferred to fresh infusion at intervals of from four to eight weeks. The clones have been obtained from various sources: from laboratory cultures, from Strawberry Creek on the Berkeley campus, from Golden Gate Park in San Francisco, from Lake Merced, and from Pacific Grove, California. In this series of experiments, a total of nine clones was used; five consisted of *Paramecium caudatum*, and four of *P. aurelia*.

In the computations for the drawing of the graphs, the division rate was averaged for five-day periods, except in figure 1, which shows the average division rate for ten-day periods.

The figures in the tables represent the means and the standard deviations with their probable errors for each of the experiments carried on under a similar set of conditions and over the same period of time. The differences between the means of the control, which with one exception (table 4) was hay infusion, and of the gland solutions are given in the tables, with the probable error of this difference. The significance factor, a term employed by Miss Phillips (1922), represents the number of times this difference of the means is a multiple of the probable error. The significance factor gives immediately the degree of significance of any difference in the average division rate. Since a difference of three times the probable error is usually taken as statistically significant, we may consider a significance factor of less than three to be the result of random sampling. A plus sign preceding the significance factor indicates an increased division rate over that of the control; a minus sign a decrease from the division rate of the control.

In determining the mean division rate, the following methods were used throughout the computations. A zero division was counted only once if the animal died without dividing even though it might live several days. To count a series of zero divisions which ended in the death of the animal would result in decreasing the division rate of the more vigorous strains; i.e., those able to live longer under pathological conditions as compared with the less vigorous ones, which die soon after ceasing to divide. Consequently, an animal was reckoned as dead as soon as it ceased dividing, even though it might not actually die for several days. A death was counted as a zero division, except when it was immediately preceded by one or more zero divisions; in the latter case, it was not counted. Zero divisions were counted, however, if the animal did not die and eventually divided. The figures given in the tables represent the total number of daily counts with the above modifications.

RESULTS

From the historical review, it may be seen that the effects of various physical and chemical agents on the division rate of ciliates have often led to contradictory results in the hands of different workers. At the time the present work was begun, however (1922), all the evidence showed that thyroid produced an acceleration in the rate of division.

Experiments with Thyroid

Experiment 1 (table 1). Division rate on hay infusion and on hay infusion plus desiccated thyroid. Bacterial factor uncontrolled.

Races 382–1, 383–1, *P. caudatum*. Race 364–1, *P. aurelia*.

The animals were transferred to freshly boiled hay infusion or to hay infusion plus thyroid. Both the control and the hay infusion plus thyroid had been previously bacterized, but all vegetative bacterial cells were killed by boiling at the time of transfer. Bacteria were transferred along with the isolated animals from the slides of the previous day, thus keeping the bacterial flora practically constant.

Neither of the two races of *P. caudatum* showed any statistically significant increase in the division rate of the thyroid-fed lines over that of the control (table 1). In race 383–1, the control had a slightly higher rate than the thyroid-fed animals; and in race 382–1, the increase with thyroid was only 1.3 times the probable error.

TABLE 1

DIVISION RATE ON HAY INFUSION AND ON HAY INFUSION PLUS DESICCATED THYROID. BACTERIAL FACTOR UNCONTROLLED

Race and date	Hay			Thyroid			Difference of means	Significance factor
	Number of counts	M	σ	Number of counts	M	σ		
382–1, *P. caudatum* June 1, 1922– Aug. 9, 1922	57	1.211 ± .064	0.713 ± .045	67	1.314 ± .060	0.732 ± .043	0.103 ± .088	+1.3
383–1, *P. caudatum* June 1, 1922– Aug. 10, 1922	50	1.385 ± .071	0.740 ± .050	67	1.310 ± .061	0.745 ± .043	0.075 ± .094	−0.8
364–1, *P. aurelia* June 11, 1922– May 26, 1923	267	1.092 ± .031	0.748 ± .022	330	1.435 ± .030	0.798 ± .021	0.343 ± .043	+8.0

On the other hand, the race of *P. aurelia* (364–1), with which this method was used for nearly a year, showed a significant increase in rate of division in the thyroid-fed line over the control (fig. 1). The mean division rate of the former was 31.4 per cent higher than that of the latter; this is about one-half of the 65 per cent acceleration in division rate found by Shumway (1917) in his thyroid-fed lines. Reference to figure 1 shows that the greatest difference between the thyroid and the control occurred during the first half of the experiment, due largely to the low division rate in the control; and that the division rates of both thyroid and control were much more alike in the latter half of the experiment.

From these data, it appeared that the race of *P. aurelia* was more susceptible to thyroid influence than the two races of *P. caudatum.* The response of this race to thyroid feeding seemed to agree with the results obtained in previous work. Nevertheless, the possibility remained that the effects were merely the result of a greater growth of bacteria in the thyroid solution; and experiments were begun in an attempt to control the bacterial factor.

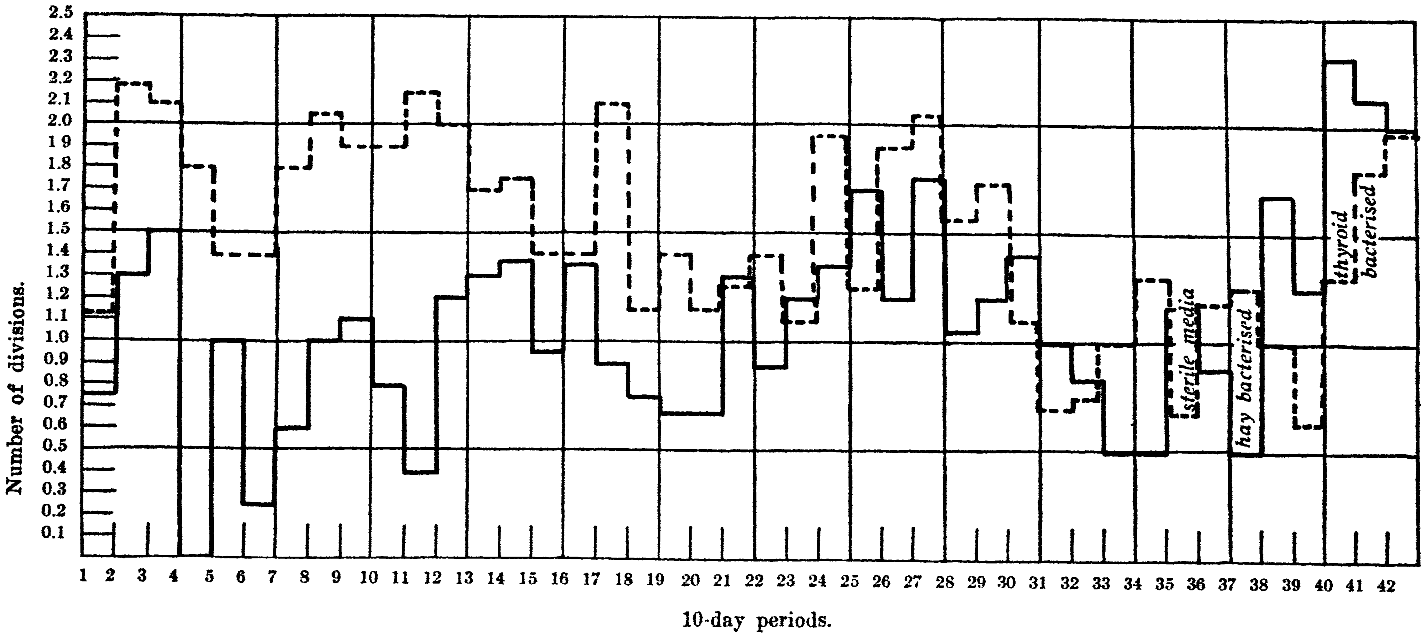

Fig. 1. Race 364-1, *P. aurelia*. Average daily division rate for ten day periods on hay infusion and on hay infusion plus thyroid. June 11, 1922, to August 3, 1923.

Bacteria uncontrolled—Periods 1–35.
Both hay and thyroid sterile—Periods 36–37.
Hay bacterized, thyroid sterile—Periods 38–40.
Both hay and thyroid bacterized—Periods 41–42.
———————— Hay infusion.
– – – – – – – Thyroid.

Experiment 2 (table 2). Bacteria the same in both thyroid and control. Media sterile at the time of transfer.

Race 364–1, *P. aurelia* (June 1, 1923–June 24, 1923).

Both the hay infusion and the hay infusion plus thyroid were autoclaved and kept sterile throughout the experiment, medium being drawn off as it was needed by means of a siphon. The bacterial food was limited to that transferred along with the isolated *Paramecium* from the slide of the previous day. Sterile hay infusion, however, was found to be quite unfavorable for the growth of *Paramecium,* as is shown by the decrease in division rate (fig. 1, periods 36 and 37) and the occurrence of 14 deaths and 6 zero divisions in 29 days.

TABLE 2

DIVISION RATE ON HAY INFUSION AND ON HAY INFUSION PLUS DESICCATED THYROID. SAME BACTERIA IN BOTH THYROID AND CONTROL. STERILE MEDIA BACTERIZED ON THE PREVIOUS DAY

Race and date	Hay			Thyroid			Difference of means	Significance factor
	Number of counts	M	σ	Number of counts	M	σ		
364–1, *P. aurelia* June 1, 1923– June 24, 1923 *	14	0.643 ± .110	0.610 ± .078	24	1.208 ± .103	0.749 ± .073	0.565 ± .151	+3.7
June 25, 1923– July 21, 1923 †	27	1.685 ± .086	0.665 ± .061	18	0.917 ± .136	0.854 ± .096	0.668 ± .161	−4.1
July 22, 1923– Aug. 3, 1923 .	13	2.096 ± .089	0.476 ± .063	13	1.846 ± .161	0.863 ± .114	0.250 ± .184	−1.4
Sept. 14, 1923– Dec. 13, 1923	162	0.761 ± .031	0.579 ± .022	155	1.142 ± .029	0.535 ± .020	0.381 ± .042	+9.1
383–1, *P. caudatum* Sept. 4, 1923– Nov. 25, 1923 . .	137	0.985 ± .037	0.636 ± .027	133	1.060 ± .037	0.634 ± .026	0.075 ± .052	+1.4

* Both hay and thyroid media sterile at the time of transfer (experiment 2)

† Hay infusion bacterized on the previous day; thyroid medium sterile at the time of transfer (experiment 3).

It was evident that, under these conditions, the thyroid-fed lines were obtaining considerably more food than the controls. Observations of the old depression slides at the time of transfer to fresh media, i.e., after one day's bacterization, showed a much heavier growth of bacteria in the thyroid medium. For some reason, *Paramecium* appears to be unable to live without food for any length of time in hay infusion on depression slides. This fact was noted many times during the course of the work and had been previously observed by Rees (1922). It evidently accounts for the high death rate of the control in this experiment.

Experiment 3 (table 2). Bacteria the same in both thyroid and the control. Control medium bacterized the day previously; thyroid medium sterile at the time of transfer.

Race 364–1, *P. aurelia* (June 25, 1923–July 21, 1923).

Since the *Paramecium* would not live on sterile hay infusion, the control was transferred to hay infusion that had been inoculated with bacteria on the previous day. In this way, it was possible to control the bacteria to a considerable extent, each inoculation being made from a slide of the previous day. The thyroid-fed line was continued on sterile medium as in Experiment 2. The immediate result was a marked increase in the division rate of the control (fig. 1, periods 38–40). At this time, the control was dividing considerably more rapidly than the thyroid-fed lines. The mean rate for the former was 1.685; and for the latter, 0.917. The difference was over four times the probable error.

Experiment 4 (table 2). Bacteria the same in both thyroid and the control. Sterile medium bacterized on the previous day.

Race 383–1, *P. caudatum.*

Race 364–1, *P. aurelia* (July 22, 1923–Aug. 3, 1923; Sept. 14, 1923–Dec. 13, 1923).

Both hay and thyroid-fed lines were placed on sterile medium bacterized on the previous day. In this experiment, the degree of bacterization was approximately the same in both lines. The division rate for both hay and thyroid was practically the same (table 2, 364–1, July 22, 1923–August 3, 1923; fig. 1, periods 40, 41), the hay infusion having a slightly higher rate. These results seemed to indicate that the acceleration in division rate with thyroid feeding was the result of an increased bacterial food supply rather than any specific effect due to the thyroid itself.

This method was continued with a race of *P. caudatum* (383–1) and with the above mentioned race of *P. aurelia* for a period of approximately three months. As shown in table 2 (race 383–1) and figure 2, there is no significant increase, in the *caudatum* race, of the thyroid-fed over the control lines.

A different result was obtained, however, with race 364–1 (*P. aurelia*), which showed a significant increase in the division rate in the thyroid over the control line (table 2, 364–1, Sept. 14, 1923–Dec. 13, 1923). It should be noted, however, that the rate of fission for the thyroid-fed line, namely, 1.142, is not excessively high, but somewhat below that for the majority of the other thyroid-fed lines. The control, on the other hand, showed an exceptionally low division rate, 0.761. Since this increase was not obtained in any other instance in experiment 4, and not at all in experiment 5, in which the bacterial factor was even more closely controlled, and since the division rate with thyroid was not accelerated while that of the control was unusually low, it is felt that this result was due to the low division rate of the control rather than to any real increase produced by feeding thyroid. Taken by itself, this experiment would show a significant increase in division rate as a result of thyroid feeding. When compared with other experiments, the acceleration is seen to be only apparent, and evidently due to some special factor working only in the particular instance.

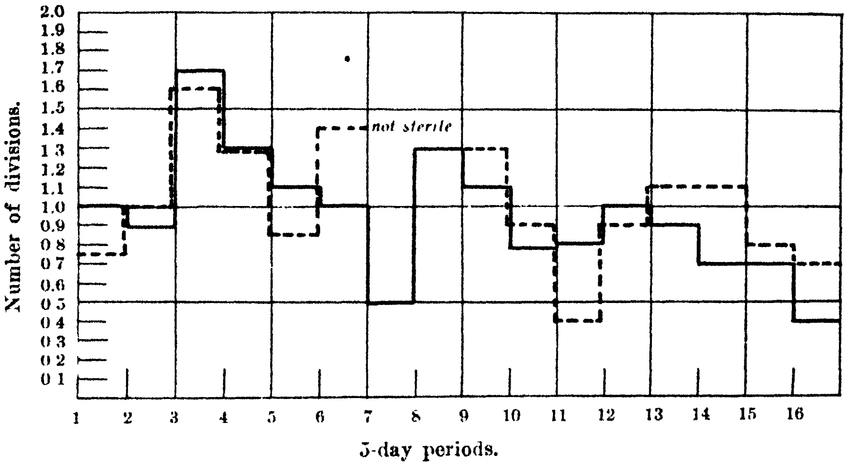

Fig. 2. Race 383–1, *P. caudatum.* Average daily division rate for five-day periods on hay infusion and on hay infusion plus thyroid. Both hay and thyroid bacterized. September 4, 1923, to November 25, 1923.

——————— Hay infusion. – – – – – – – Thyroid.

Experiment 5 (table 3). Both thyroid and control fed on the same kind of dead bacteria.

Races 385–1, 390–1, 48–2, *P. caudatum.*

Races 364–1, 250–1, 389–1, 393–1, *P. aurelia.*

Although Peters (1921) has been able to cultivate *Colpidium* on bacteria-free medium, it has not yet been possible to raise *Paramecium* on sterile food. However, it was found that these ciliates could live on dead bacteria plus the few bacteria carried over with the animals at the time of transfer. Hay infusion was inoculated with bacteria from a flourishing culture, care being taken that no *Paramecium* was transferred at the same time. When the culture had developed a rich bacterial flora, it was autoclaved, and two drops of the sterile bacterized infusion added each day to the depression slide. Seven different clones were used; none of them showed a statistically significant increase for the thyroid-fed lines (table 3; figs. 3 and 4). One of these clones of *P. aurelia* (364–1) was used in experiment 4, in which the thyroid-fed line showed a significant increase over the control

TABLE 3

DIVISION RATE ON HAY INFUSION AND ON HAY INFUSION PLUS DESICCATED THYROID. BOTH THYROID AND CONTROL ON SAME KIND OF DEAD BACTERIA

Race and date	Hay			Thyroid			Difference of means	Significance factor
	Number of counts	M	σ	Number of counts	M	σ		
385–1, *P. caudatum* Dec. 15, 1923–Mar. 2, 1924	144	1.281 ± .029	0.525 ± .021	147	1.327 ± .041	0.730 ± .029	0.046 ± .050	+0.9
390–1, *P. caudatum* Feb. 6, 1924–Feb. 29, 1924	43	1.140 ± .055	0.532 ± .039	30	1.295 ± .075	0.695 ± .053	0.155 ± .093	+1.7
48–2, *P. caudatum* Feb. 8, 1924–Apr. 3, 1924	105	1.150 ± .044	0.665 ± .031	101	1.262 ± .048	0.720 ± .034	0.112 ± .065	+1.7
364–1, *P. aurelia* Dec. 11, 1923–Jan. 28, 1924.	74	0.932 ± .048	0.617 ± .034	76	1.053 ± .053	0.691 ± .038	0.121 ± .072	+1.7
250–1, *P. aurelia* Feb. 14, 1924–Apr. 3, 1924	60	0.933 ± .057	0.649 ± .040	86	0.988 ± .051	0.707 ± .036	0.055 ± .076	+0.7
389–1, *P. aurelia* Feb. 8, 1924–Apr. 3, 1924	101	1.275 ± .043	0.642 ± .030	101	1.327 ± .041	0.615 ± .029	0.052 ± .059	+0.9
393–1, *P. aurelia* Feb. 25, 1924–Apr. 3, 1924	62	0.645 ± .051	0.592 ± .036	63	0.746 ± .054	0.635 ± .038	0.101 ± .074	+1.4

(fig. 3). The failure of this line to show any increase under more carefully controlled bacterial conditions points to the probability that some unknown factor, perhaps an unfavorable strain of bacteria, was responsible for the results in experiment 4.

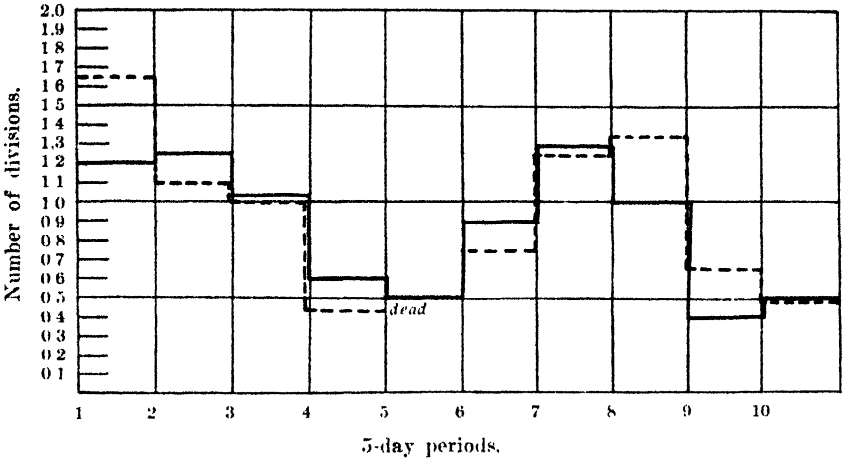

Fig. 3. Race 364-1, *P. aurelia.* Average daily division rate for five-day periods on hay infusion and on hay infusion plus thyroid. Both hay and thyroid lines fed on dead bacteria. December 11, 1923, to January 28, 1924.

———— Hay infusion.

- - - - - Thyroid.

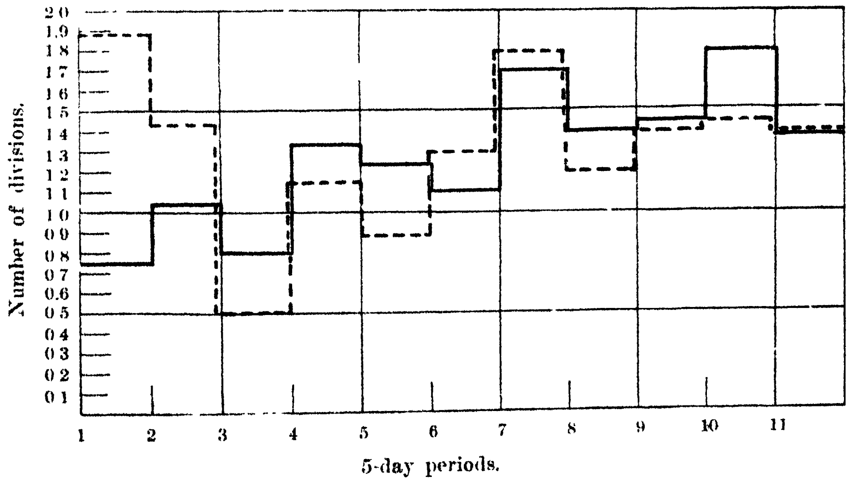

Fig. 4. Race 389-1, *P. aurelia.* Average daily division rate for five-day periods on hay infusion and on hay infusion plus thyroid. Both hay and thyroid lines fed on dead bacteria. February 8, 1924, to March 2, 1924.

———— Hay infusion.

- - - - - - Thyroid.

EXPERIMENTS WITH SOLUTIONS OF PITUITARY AND LIVER

Experiment 6 (table 4). Division rate on hay infusion and on hay infusion plus pituitary, thyroid, or liver.

Race 364–1, *P. aurelia.*

Solutions of the anterior and posterior lobes of fresh beef hypophysis were added to the hay infusion and produced a marked acceleration in division rate over the control, an increase in many cases greater than that produced by thyroid. When compared with the rate on solutions of fresh liver, however, this acceleration disappeared. In many instances, the strain fed on liver divided faster than that fed on hypophysis (table 4). There is little doubt that, in this case, the increased division rate was the result of a more favorable food supply and not the specific effect of any substance secreted by the endocrine gland.

In order to test still further the effect of thyroid, two drops of thyroid solution were added to each of the foregoing gland solutions. In no case did there result a significant increase in the division rate (table 4). This is additional evidence that the effect of the thyroid is merely to provide a better bacterial food supply than is afforded by hay infusion alone. It is possible to substitute a solution of liver for that of thyroid and to obtain as great an increase in the rate of division.

EXPERIMENTS WITH GLYCOGEN

Experiment 7 (table 5). Division rate on hay infusion and on hay infusion plus glycogen.

Races 383–1, 385–1, 390–1, 48–2, *P. caudatum.*

Races 364–1, 250–1, 389–1, 393–1, *P. aurelia.*

There is evidence that glycogen is used up in the process of division in certain Protozoa (Zweibaum, 1922; Kofoid and Swezy, 1921). It was possible that the acceleration in division rate produced by the solution of liver might have been the result of the glycogen present. Experiments using powdered glycogen were conducted for over five months with eight different clones of *Paramecium.* The results are shown in table 5. Glycogen powder in strengths of 0.5 mg. to 1.0 mg. per cc. produced a marked decrease in division rate. A dilution of

TABLE 4

DIVISION RATE ON HAY INFUSION AND ON HAY INFUSION PLUS PITUITARY, THYROID, OR LIVER

Race 364-1, *P. aurelia*

	Hay		Liver		Anterior lobe		Posterior lobe		Liver plus thyroid		Anterior lobe plus thyroid		Posterior lobe plus thyroid		Thyroid *	
	M	σ	M	σ	M	σ	M	σ	M	σ	M	σ	M	σ	M	σ
Jan. 24, 1923–May 26, 1923	1.210±.048	0.736±.034	1.611±.062	0.858±.044	1.435±.041	0.611±.029	1.509±.058	0.905±.041	1.616±.054	0.793±.038	1.456±.062	0.880±.044	1.540±.067	0.982±.047		
Number of counts	106		88		103		111		97		91		99			
Difference of means			0.401±.078		0.225±.063		0.299±.075		0.406±.072		0.246±.078		0.330±.082			
Significance factor			+5.3		+3.6		+4.0		+5.6		+3.2		+4.0			
	M	σ	M	σ	M	σ	M	σ	M	σ	M	σ	M	σ	M	σ
May 27, 1923–June 24, 1923	0.643±.110	0.610±.078	1.037±.097	0.744±.068	1.077±.097	0.730±.068	1.200±.101	0.748±.071	1.324±.104	0.799±.073	0.963±.075	0.576±.053	1.250±.060	0.472±.043		
Number of counts	14		27		26		25		27		27		28			
Difference of means			0.394±.147		0.434±.147		0.557±.149		0.681±.151		0.320±.133		0.607±.125			
Significance factor			+2.7		+2.9		+3.7		+4.5		+2.4		+4.9			
	M	σ	M	σ	M	σ	M	σ	M	σ	M	σ	M	σ	M	σ
June 25, 1923–Aug. 3, 1923	1.819±.068	0.640±.048	2.038±.046	0.435±.033	2.224±.070	0.652±.050	2.228±.095	0.895±.067	2.235±.087	0.744±.062	2.111±.074	0.655±.052	1.524±.092	0.763±.065		
Number of counts	40		40		39		40		33		36		31			
Difference of means			0.219±.082		0.405±.098		0.409±.117		0.416±.110		0.292±.100		0.295±.114			
Significance factor			+2.7		+4.1		+3.5		+3.8		+2.9		−2.6			
	M	σ	M	σ	M	σ	M	σ	M	σ	M	σ	M	σ	M	σ
Jan. 24, 1923–Aug. 3, 1923			1.621±.044	0.817±.031	1.563±.045	0.859±.032	1.629±.048	0.946±.034	1.696±.045	0.839±.032	1.523±.047	0.869±.033	1.486±.047	0.877±.033	1.367±.049	0.779±.035
Number of counts			155		168		176		157		154		158		113	
Difference of means (*Liver-control*)					0.059±.063		0.008±.065		0.075±.063		0.098±.064		0.135±.064		0.254±.066	
Significance factor					−0.9		+0.1		+1.2		−1.6		−2.1		−3.9	

* Thyroid from Jan 24, 1923 to May 26, 1923, only.

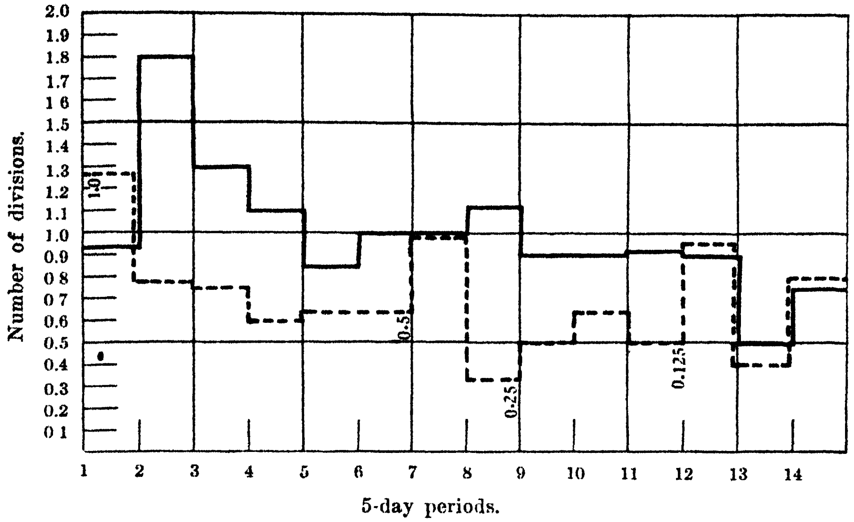

Fig. 5. Race 383–1, *P. caudatum.* Average daily division rate for five-day periods on hay infusion and on hay infusion plus glycogen. Both hay and glycogen lines fed on dead bacteria. September 4, 1923, to November 19, 1923.

1.0 mg. glycogen per cc.—Periods 1–6.
0.5 mg. glycogen per cc.—Periods 7–8.
0.25 mg. glycogen per cc.—Periods 9–11.
0.125 mg. glycogen per cc.—Periods 12–14.
——— Hay infusion. – – – – – – Glycogen.

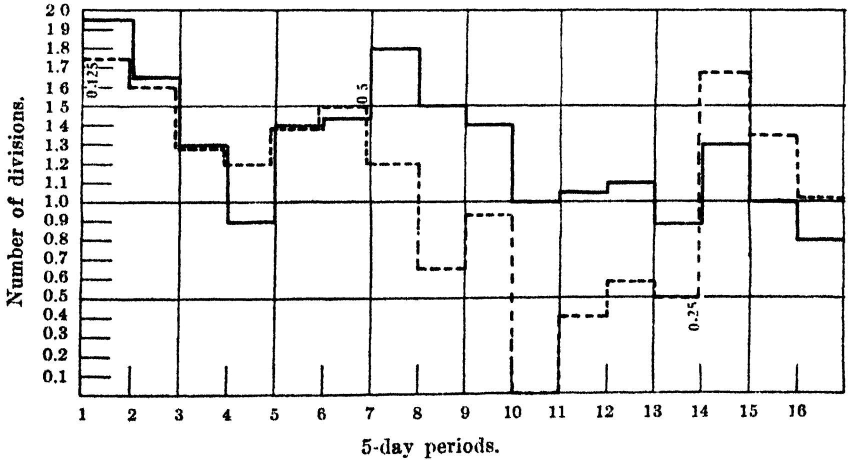

Fig. 6. Race 385–1, *P. caudatum.* Average daily division rate for five-day periods on hay infusion and on hay infusion plus glycogen. Both hay and glycogen lines fed on dead bacteria. December 15, 1923, to March 2, 1924.

0.125 mg. glycogen per cc.—Periods 1–6.
0.5 mg. glycogen per cc.—Periods 7–13.
0.25 mg. glycogen per cc.—Periods 14–16.
——— Hay infusion. – – – – – – Glycogen.

0.25 mg. usually had no significant effect on the rate of fission; in certain cases, however (383–1, *P. caudatum,* and 364–1, *P. aurelia*), solutions of this strength also produced a noticeable lowering of the division rate. The clone of *P. aurelia* (364–1) was also affected by a dilution of as low as 0.125 mg. per cc., but not by a strength of 0.0625 mg. per cc. Dilutions of less than 0.25 mg. per cc. did not affect the division rate of the other clones tested. In no instance did glycogen produce a significant increase in the rate of division. In all cases, the bacterial factor was controlled, either by using sterile medium bacterized on the previous day or by adding dead bacteria to the sterile medium.

EXPERIMENTS ON METABOLIC RATE

Experiment 8 (table 6). Effect of desiccated thyroid on volume of oxygen consumed.

Race 385–1, *P. caudatum.*

An attempt was made to measure the metabolic rate directly by means of a Thunberg-Winterstein microrespirometer (Thunberg, 1905; Winterstein, 1912). This apparatus was used to measure the metabolic rate of *Paramecium* by Zweibaum (1921). Essentially, it consists of two glass chambers connected by a glass tube having a 2 mm. bore. Each chamber has a volume of about 3 cc. Equal volumes of culture fluid are placed in each chamber; one contains *Paramecium,* together with the bacteria on which it is feeding; the other contains the bacteria without the *Paramecium.* The CO_2 given off is absorbed by concentrated NaOH, suspended in dishes over the two cultures. The difference in the amount of oxygen absorbed in the two chambers represents the volume of oxygen used up by the *Paramecium.* This can be determined by measuring the movement of an oil droplet in the connecting tube. By using large numbers of animals, it is possible to obtain a movement of the oil droplet of several millimeters in a few hours.

Although the results are not so complete as might be wished, they show no marked increase in the volume of oxygen consumed by the animals living in thyroid solution, compared to the control on hay infusion (table 6). In other words, the evidence, although based on only a few cases, shows no increase in the metabolic rate, as measured by oxygen consumed, due to thyroid feeding.

TABLE 5

DIVISION RATE ON HAY INFUSION AND ON HAY INFUSION PLUS GLYCOGEN

Race and date	Mgs per cc	Hay			Glycogen			Difference of means	Significance factor
		Number of counts	M	σ	Number of counts	M	σ		
383-1, *P. caudatum* Sept 4, 1923-Oct. 9, 1923	1 0	61	1 115 ± 056	0 649 ± 040	55	0 809 ± 056	0 614 ± .039	0 306 ± 079	−3 9
364-1, *P aurelia* Sept. 16, 1923-Oct. 9, 1923	1 0	38	1 072 ± 075	0 681 ± 053	26	0.615 ± 064	0 487 ± 046	0 457 ± 099	−4 6
383-1, *P caudatum* Oct 10, 1923-Oct 20, 1923	0 5	18	1 000 ± 091	0 574 ± 065	13	0 462 ± 093	0 498 ± 066	0 538 ± 130	−4 1
385-1, *P caudatum* Jan 13, 1924-Feb 15, 1924	0 5	64	1 250 ± 057	0 679 ± 040	46	0 793 ± 068	0 688 ± 048	0 457 ± 089	−5 1
364-1, *P aurelia* Oct 10, 1923-Oct 20, 1923 Jan 11, 1924-Jan 16, 1924	0 5	28	0 911 ± 080	0 628 ± 057	23	0 522 ± 070	0 499 ± 050	0 389 ± 106	−3 7
383 1, *P caudatum* Oct 21, 1923-Nov 1, 1923	0 25	22	1 046 ± 110	0 767 ± 078	20	0 550 ± 075	0 497 ± 053	0 496 ± 133	−3 7
385-1, *P caudatum* Feb 15, 1924-Mar 2, 1924	0 25	31	1 032 ± 094	0 772 ± 066	29	1 319 ± 089	0 679 ± 060	0 287 ± 129	+2 2
390 1, *P caudatum* Feb 7, 1924-Mar. 1, 1924	0 25	13	1 140 ± 055	0 532 ± 039	28	1 107 ± 092	0 724 ± 065	0 032 ± 107	−0.3
48-2, *P. caudatum* Feb 8, 1924 Apr 3, 1924	0 25	105	1 150 ± 044	0 665 ± 031	90	1 122 ± 050	0 708 ± 036	0 028 ± 067	−0 4
364-1, *P aurelia* Oct 21, 1923-Oct 31, 1923	0 25	22	0 864 ± 076	0 526 ± 053	23	0 565 ± 070	0 496 ± 049	0 299 ± 103	−2 9
250 1, *P aurelia* Feb 8, 1924 Apr 3, 1924	0 25	60	0 933 ± 057	0 649 ± 040	58	1 051 ± 056	0 628 ± 039	0 118 ± 080	+1 5
389-1, *P aurelia* Feb 8, 1924-Apr 3, 1924	0 25	101	1 275 ± 043	0 642 ± 030	98	1 287 ± 045	0 663 ± 032	0 012 ± 062	+0 2
393 1, *P aurelia* Feb 25, 1924-Apr 3, 1924	0 25	62	0 645 ± 051	0 592 ± 036	43	0 605 ± 059	0 576 ± 042	0 041 ± 078	−0 5
383-1, *P. caudatum* Nov. 5, 1923 Nov. 19, 1923	0 125	30	0 733 ± 054	0 442 ± 038	33	0 636 ± 062	0 526 ± 044	0.097 ± 082	−1 2
385-1, *P caudatum* Dec 10, 1923-Jan. 12, 1924	0 125	49	1 479 ± 057	0 589 ± 040	56	1 446 ± 059	0 652 ± 042	0 033 ± 082	−0.4
364-1, *P. aurelia* Nov. 1, 1923-Nov. 27, 1923 Dec 10, 1923-Jan 11, 1924.	0 125	101	0 812 ± 037	0.553 ± 026	83	0 639 ± 044	0 593 ± 031	0 173 ± 057	−3.0
364-1, *P. aurelia* Nov. 28, 1923-Dec 11, 1923	0.0625	28	0 607 ± 071	0 557 ± 050	23	0 696 ± 077	0 546 ± 054	0 089 ± 105	+0 8

TABLE 6

EFFECT OF DESICCATED THYROID ON THE VOLUME OF OXYGEN CONSUMED

Race 385–1, *P. caudatum*

Date	Medium	Volume of fluid	Time of experiment	Number of *Paramecium*	Volume of oxygen consumed in mm.^{3} per hour per 1,000 *Paramecium*
Feb. 20, 1924	Hay	0.64 cc.	6 hours	17,400	0.511
Feb. 21, 1924	Thyroid	0.64 cc	7 hours	17,400	0.542
March 19, 1924	Hay	0.64 cc.	5 hours	8,800	0.605
March 24, 1924	Thyroid	0.64 cc.	4 hours	10,800	0.614

GENERAL DISCUSSION

The symptoms of hyperthyroidism in the Mammalia and the Amphibia are well known. They are the result, in the main, of an increased metabolic rate. On the other hand, Rémy (1922) and Mason (1923) were unable to accelerate the metamorphosis of *Petromyzon* larvae by use of thyroid. Furthermore, the effect of thyroid on invertebrates appears to be negligible in most respects. Kunkel (1918) and Kahn (1921) found no marked acceleration in the development of insects; and Wulzen (1923) could demonstrate no increase in growth or fission rate in *Planaria*.

In addition, even in such forms as tadpoles, which undergo marked structural changes as the result of thyroid feeding, there is no evidence that increased metabolic rate necessarily manifests itself in accelerated cell division. Champy (1922) has shown in his histological studies of the tissues of thyroid-fed tadpoles, that, while certain regions of the body are characterized by large numbers of dividing cells, other regions such as the tail are unaffected or may even divide less rapidly than in normal animals. The effect of the thyroid seems to be selective, acting on different tissues in different ways.

It is only in the Protozoa that it has been assumed that an increase in metabolic rate would manifest itself as an increase in the rate of cell division. Neverthless, metabolic rate might be doubled, but if both the anabolic and the katabolic processes were increased in the same proportion, the division rate would remain unchanged. Or, if the katabolic processes were increased in greater proportion than the anabolic, it is even possible that a higher metabolic rate would result in decreased cell multiplication.

Furthermore, the failure to obtain any marked result by the use of thyroid in the lower vertebrates or in the invertebrates would point to the fact that the thyroid hormone does not act merely on the cells, as such, but that it produces its effect by a much more complex mechanism. The conditions under which the gland can alter the rate of metabolism are present in some, mostly the higher, organisms; and are absent in other, mainly the lower, organisms.

It is not surprising, therefore, that thyroid, solely as a hormone, fails to produce an acceleration in the division rate of *Paramecium*. Thyroid is an exceptionally suitable food for *Paramecium;* recently Straus (1923) has recommended it as superior to hay infusion for stock cultures. The value of thyroid as a source of food for *Paramecium* is that it provides excellent opportunities for the growth of bacteria.

Another factor which must be taken into account is that different clones of the same species of Protozoa respond differently to the same conditions. It is necessary to use more than a single clone before drawing any general conclusions. It is possible that this fact is the explanation of many of the contradictory results that have been found in the past in experimental work on the Protozoa.

For the clones tested in this investigation, the proof that the acceleration in division rate is the result of a more favorable food supply rests on the following experimental evidence. (*a*) When bacteria were allowed to multiply for several days in both the hay infusion and the thyroid, the latter produced a marked acceleration in division rate, although not in all clones (table 1). (*b*) Providing the control with media bacterized on the previous day, while keeping the thyroid-fed line on media bacterized only at the time of transfer, produced a much higher division rate in the control than in the thyroid line (table 2). (*c*) The use, in both the hay and the thyroid lines, of sterile media bacterized on the previous day with bacteria from a common source produced no marked acceleration in the division rate of the thyroid-fed strain (table 2). (*d*) The use of dead bacteria as the principal source of food for both lines gave no significant increase with thyroid (table 3). (*e*) The acceleration produced by thyroid with uncontrolled bacterial conditions was even lower than that produced under similar conditions by liver or by hypophysis (table 4). (*f*) The addition of thyroid to solutions of liver or hypophysis, in which the bacterial flora was already very rich, caused no significant increase in the division rate over the same solutions without thyroid (table 4).

The failure to demonstrate directly any increase in metabolic rate (table 6), although based on only a few experiments, is in line with the above mentioned results on invertebrates and on cyclostomes.

Because of its control over growth, it might be thought that the pituitary would produce a marked effect either upon the size or upon the division rate of *Paramecium.* There is no evidence that there is any significant difference in size or division rate between pituitary-fed lines and the control fed on liver solution. This was found to be true both for the anterior and the posterior lobes. Wulzen (1916), however, using *Planaria,* with liver as a control, was able to produce a marked increase in size by feeding anterior lobe, and a marked increase in the rate of fission by feeding any part of the pituitary. We may repeat what was said regarding the thyroid: the final effect depends both upon the substance used and upon the organism tested. Conclusions proved for one organism or for one group of organisms cannot be applied widely to all organisms without adequate experimentation.

The reason for the marked decrease in division rate in the stronger solutions of glycogen is not known. The glycogen used was obtained from two different sources; it is doubtful if this effect is due to any contamination present. The bacterial factor was controlled in all the glycogen experiments. If strong solutions of glycogen are harmful, weaker ones are without any effect on the division rate. There is no evidence that the increase produced by the liver solution is due to the glycogen present (table 5).

SUMMARY

For the clones tested:

1. With an uncontrolled bacterial food supply, members of the same clone of *Paramecium* may divide at a significantly higher rate in solutions of desiccated thyroid than in the hay infusion used as a control.

2. By increasing the bacterial food supply of the control, it is possible to bring its division rate considerably above that of the thyroid-fed line.

3. When approximately equal numbers of bacteria are provided for both lines, there is no significant increase in the division rate of the thyroid-fed *Paramecium.*

4. The acceleration in division rate produced with thyroid and an uncontrolled bacterial food supply is no greater than that obtained under similar conditions with solutions of liver or hypophysis.

5. Addition of thyroid to solutions of liver or hypophysis produces no significant increase in the rate of division.

6. All the experimental evidence in this investigation points to the fact that thyroid accelerates the division rate of *Paramecium* by providing a favorable bacterial food supply, and not by any specific action of the thyroid hormone.

7. Solutions of both the anterior and the posterior lobes of the pituitary gland produce no significantly higher rate of division than does a solution of liver.

8. Glycogen does not accelerate the rate of cell division, but decreases it in strong solutions and is without any effect in weaker ones.

9. By direct measurement, no increase in the metabolic rate could be demonstrated in the thyroid-fed individuals over the rate in the controls.

10. Different clones show considerable variation in their response to similar conditions.

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STUDIES ON PARAMECIUM

II. THE BEHAVIOR OF A CONJUGATING RACE OF PARAMECIUM CAUDATUM

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INTRODUCTION

In any genetic study of ciliates, it is essential that the process of conjugation be more or less accurately controlled. In *Paramecium*, various methods have been recommended for insuring conjugation, but no one of them has proved successful under every condition; and the methods recommended by one worker have frequently failed when employed by others. The remarkable results of Zweibaum (1912) who obtained practically 100 per cent conjugation in *Paramecium* by the use of appropriate dilutions of favorable electrolytes suggested a possible method of obtaining conjugants at will. Taking these experiments as a point of departure, attempts were made by the writer to control the process of conjugation, instead of relying upon the fortuitous and comparatively infrequent epidemics which occur under normal conditions. The results have been essentially negative, but the attempt has been marked by the discovery of a race of *Paramecium caudatum* which conjugated in practically every instance when the proper conditions were supplied. A fairly thorough study of this race has been carried out in an effort to determine the factors governing its behavior and to compare them with the conditions for conjugation which have proved favorable for other races.

ACKNOWLEDGMENTS

The experiments described in this paper form the second of a series of studies on *Paramecium.* They have been carried out under the direction of Professor C. A. Kofoid, to whom the writer is greatly indebted for many helpful suggestions and criticisms. The writer also wishes to thank Professor T. D. Beckwith for extending the facilities of the laboratory of the Department of Bacteriology. A considerable portion of the work was carried on while the writer was holding the University Fellowship in Zoology for 1923–1924.

HISTORICAL REVIEW

The literature bearing on the problem of the conditions for conjugation in ciliates has been well reviewed by Dobell (1914) and by Jennings (1920). It will be considered here only as it bears directly upon the experiments described in the present paper.

Maupas (1889) maintained that the process of conjugation was entirely independent of external factors. According to him, the conditions required for conjugation are (1) starvation; (2) karyogamic maturity; (3) exogamy. Later work has proved that, while these conditions may be necessary in some races, conjugation may occur in other races independently of any of them.

Regarding karyogamic maturity, Joukowsky (1898) showed in *Paramecium putrinum* that the descendants of an ex-conjugant could conjugate among themselves, even after seven or eight divisions, without any ill effects; and conjugation between organisms even less far removed from a previous conjugation has been described by Jennings (1910), by Enriques (1916), and by Klitzke (1914). Nevertheless, there is usually an interval of several days between one epidemic of conjugation and the next, even with those lines that show the most pronounced tendency toward frequent conjugation (Jennings, 1910; Calkins and Gregory, 1913; Calkins, 1919; Hopkins, 1921; Woodruff and Spencer, 1924).

Endogamy rather than exogamy appears to be the rule in ciliates, at least in laboratory cultures. There seems to be no authentic recorded instance of conjugation between ciliates belonging to different clones. The closest approach to exogamy is a case recently described by Woodruff and Spencer (1924) in which they were able to cross two individuals which belonged to the same clone of *Spathidium*, but had been kept in separate cultures and were removed from a common ancestor by 982 divisions.

Maupas' third requirement, hunger, has been the method used most successfully in bringing about conjugation. Starvation does not, in itself, result in conjugation, but if the organisms are transferred from a rich, well-fed culture to one in which the food supply is rapidly exhausted, conjugation frequently, but not always, occurs.

On the other hand, Hance (1917) was able to induce conjugation in *Paramecium* by allowing the medium to evaporate; in this case there was no sudden transfer to unfavorable food conditions but a much more gradual exhaustion of food. Jennings (1910) found a race of *P. aurelia* that conjugated not as a result of starvation, but, as he maintained, at the beginning of a decline in rich food conditions; and Enriques (1916) described similar behavior in a race of *Chilodon uncinatus*. Kasanzeff (1901) stated that conjugation occurred during an increase and not during a decrease in the division rate, citing instances of so-called "hunger divisions" preceding conjugation. Calkins (1902) found that transfer to distilled water tended to inhibit rather than to aid conjugation; and Hopkins (1921) found several races of *Paramecium* that conjugated better when well fed than when starved.

Enriques (1907, 1909, 1910, 1916) and Zweibaum (1912) have been the chief proponents of the theory that the conditions favorable for conjugation are purely external. Enriques (1907) found that *Colpoda steini* conjugated only when the culture fluid reached a depth of 2–3 mm. With *Cryptochilum nigricans* (1909), he found that the addition of slight amounts of ferric chloride or of the halogen salts of sodium greatly increased the number of conjugants. Enriques stated (1910) that sometimes he was able to produce conjugation in a non-conjugating culture by the addition of fluid from a conjugating culture; and to stop conjugation in the latter by the addition of culture fluid from the former. In *Chilodon uncinatus* (1916), he was able to induce conjugation among the ex-conjugants merely by the addition of fresh food.

Zweibaum (1912), working with animals belonging to a single clone of *P. caudatum,* was able to induce conjugation by the following method. The organisms were transferred from stock cultures to crystallizing dishes to which no fresh hay had been added. These formed the starvation-cultures (*cultures de disette*). After remaining five weeks or longer under starvation conditions, the animals were placed on fresh hay infusion and transferred to new medium every three or four days (*cultures continuatives*). As long as this transferring continued, the organisms were prevented from conjugating in these cultures, but they retained the power to conjugate when subjected to the so-called *cultures d'expériences.* On about the third day after transfer to fresh medium, 5 cc. of the *Paramecium*-culture were added to 15 cc. of an appropriate dilution of certain salts. When the foregoing conditions were fulfilled, conjugation always occurred in from sixteen to twenty-four hours. With $AlCl_3$, approximately 100 per cent conjugation was obtained; while $FeCl_3$, $NaNO_3$, and Na_2CO_3 gave 75–80 per cent conjugation. No such results were obtained if the cultures had not been starved for five weeks or longer, or if the concentrations of the salts were too high or too low, or if the ciliates were transferred to distilled water to which no salts had been added.

Although Zweibaum (1912) stated that these results could be obtained with any race of *Paramecium,* and has been able to obtain them again after an interval of nine years (1921), other workers (Jollos, 1921; Hopkins, 1921) have not succeeded in confirming his experiments. Hopkins found considerable variation among different clones in ability to conjugate and in the nature of response to the conditions proposed by Zweibaum. In none of the races tested by Hopkins was he able to induce conjugation to so great an extent or with so high a degree of certainty as did Zweibaum. Furthermore, he found that conjugation frequently occurred as strongly in the *cultures continuatives* as in the smaller cultures to which salts had been added. Addition of the salts directly to the *cultures continuatives* frequently increased the percentage of conjugation; and, in some races, the cultures which were renewed with hay infusion alone conjugated more strongly than those subjected to salt solutions.

Although the greater part of Hopkins' work was performed with $NaNO_3$, his results with salts in which the gram-equivalent and the gram-molecular-weight of the solute are different are open to a possible objection. Zweibaum (1912) defines a normal solution as one containing the molecular weight of the salt dissolved in one liter (p. 360)—a statement which he alters in his 1921 paper by substituting the letter *m* for *n* in designating the strengths of the solutions used (p. 103). Consequently, in Hopkins' work, the normal solutions of such salts as $AlCl_3$ and $FeCl_3$ are only one-third as strong as those designated similarly in Zweibaum's paper. Although, even at this dilution, these salts enhance the power to conjugate, according to Zweibaum, yet they do not increase it to so great an extent as do the stronger solutions.

Chatton and Chatton (1923*a*, *b*) found that *Glaucoma scintillans* would always conjugate on medium containing a favorable dilution of certain salts, such as $CaCl_2$, provided that the ciliates were fed *Bacillus coli*; but that they never conjugated if they were feeding on *B. fluorescens*. On the other hand, *Colpidium colpoda* would conjugate with the appropriate salts in the presence of *B. fluorescens*, but not if they were fed with *B. coli*. They found that conjugation occurred in cultures that were well fed and that it was not the result of starvation, since it ensued twenty-four to thirty-six hours before the ciliates ceased dividing.

MATERIAL AND METHODS

The animals used in this experiment were members of single clones of *Paramecium aurelia* and of *Paramecium caudatum*, except in experiment 2, which was carried out with mass cultures. The clones used were derived from various sources; most of them had been carried for over a year in pure culture before being tested, and some had been previously maintained, for an indefinite period, in laboratory cultures. The history of each clone, so far as it is known, is given in detail in experiment 3.

The earlier experiments were carried out at room temperatures. Beginning with experiment 3, all the cultures were kept in an electrically regulated incubator at a temperature of 22°–24° C. Unless otherwise noted, the cultures were carried in covered crystallizing dishes. These were of two sizes: 10 cm. in diameter by 5 cm. high; and 15 cm. in diameter by 7.5 cm. high. The latter were of the size used by Zweibaum. For tests with salt solutions or with distilled water, small stender dishes 5 cm. in diameter and 3 cm. high were employed, approximately the same size as those of Zweibaum. The intensity of conjugation did not appear to be influenced by the size of these containers. A few of the main cultures were carried in the small stender dishes. Large Petri dishes, 11 cm. in diameter, were found not to be so favorable for conjugation. Because they were easily handled and provided sufficient numbers of animals for experimentation, the crystallizing dishes 10 by 10 by 5 cm. were used in the great majority of cases for the *cultures continuatives,* or, as they will be called in the future, the *serial cultures.*

At each transfer, 100–150 cc. of fresh media were placed in a crystallizing dish. To this were added from 20 to 50 cc. of the old culture medium, containing *Paramecium.* In the experiments with salts and distilled water, 5 cc. of the culture fluid containing the ciliates were added to 15 cc. of the solution to be tested. These volumes are the same as those used by Zweibaum under similar conditions. When it was seen, however, that the volume of the culture fluid was not influencing the course of conjugation, from 200 to 300 cc. of fresh medium were used with the largest-sized crystallizing dishes. The Petri dishes would not hold more than 80–100 cc. of the medium. Since most of the serial cultures were carried in the medium-sized crystallizing dishes, 20–50 cc. of culture fluid were added to 120–150 cc. of fresh medium in the majority of transfers; the liquid in such a culture had a depth of about 3 cm.

Unless otherwise stated, the medium used was fresh hay infusion. This was prepared by boiling 5 grams of timothy hay in 1 liter of distilled water from 3 to 5 minutes. If the infusion is boiled longer, the medium becomes too acid upon the multiplication of bacteria, and the whole culture will die out or suffer great reduction in numbers on the first to the third day after transfer. A culture not entirely killed by the excessive acidity may frequently be saved by dilution with distilled water or by the addition of some magnesium carbonate or of pieces of marble. In the small stender dishes, the fluid is apparently shallow enough to prevent the development of a harmful degree of acidity.

The cultures were examined daily under a binocular microscope with a magnifying power of sixteen. It was possible to look over the entire dish and to discover practically all the conjugating pairs present. The number of animals in the dish each day, as determined by an arbitrary grading scale, was noted on the record sheet, together with the occurrence of conjugation and the approximate percentage of conjugating pairs. The entire history of the conjugating race from September, 1923, to April, 1924, was recorded on a large chart, from which the majority of tables and graphs have been derived.

Cultures from ex-conjugants were obtained by transferring a conjugating pair to a depression slide and isolating the two ex-conjugants upon separation. The four individuals produced by the first two divisions of each ex-conjugant were isolated in turn and transferred first to hanging-drop slides and then to small stender dishes, until they had multiplied sufficiently to be used for conjugation tests. It was essential to provide plenty of food at all times for both conjugants and ex-conjugants, or they would not successfully complete the process of reorganization.

RESULTS

PRELIMINARY OBSERVATIONS

In May and June, 1922, nineteen clones were isolated; and cultures derived from them were carried in quarter-pint milk bottles, the maximum depth of the fluid being eight centimeters. These cultures were observed daily for a period of approximately four months; and records were kept of the numbers of *Paramecium,* and of the occurrence and extent of conjugation. The cultures were transferred to fresh food every three to four weeks. These observations (table 1) showed that certain clones were much more apt to conjugate than others; that there appeared to be no evidence for the occurrence of a cycle of conjugation at regular intervals; and that there was no noticeable correlation between the occurrence of a period of conjugation and the numbers of animals in the culture immediately following this period; i.e., an epidemic of conjugation did not seem to have any marked effect on the division rate. Most of the instances of conjugation occurred from one to two weeks after the animals were transferred to fresh medium, probably at a time when the food conditions were beginning to decline. Conjugation very rarely occurred in an old culture, in which the organisms were few and ill fed. Race 385–1, which later proved to have exceptional powers of conjugation, did not exhibit any marked tendency to conjugate during the preliminary observations.

TABLE 1

PRELIMINARY OBSERVATIONS OF FREQUENCY OF CONJUGATION. CULTURES OBSERVED DAILY. MAY-DECEMBER, 1922

Race, Date culture started	Number consecutive daily records	Days after feeding that conjugants appeared	Number of animals at time of conjugation
P. caudatum			
48–1			
May 30	74	14, 16	Constant
June 6	77	8–9	Constant
Aug. 26	44	7–11	Constant
Sept. 24	41	4	Constant
Dec. 3	...	7	...
48–2			
May 30	120	12–19	Decreasing
		61, 63–64, 66	Constant
June 6	43	11, 13	Increasing
Aug. 2	68	9–10, 38	Constant
Nov. 12	...	...	...
Dec. 3	...	...	...
48–3			
May 30	109	...	...
June 6	118	...	...
Sept. 30	20	...	...
Oct 15	16	...	...
360–5			
May 30	74	...	...
June 6	54	8, 10	Constant
Aug. 2	53	7, 10	Constant
Aug. 26	45	4	Increasing
Sept. 24	23	5, 6	Increasing
382			
May 30	74	17–18	Constant
June 6	89	11–12	Increasing
Sept. 2	33	7, 9	Constant
383–1			
May 30	74	...	
June 6	119	...	...
Aug. 26	38	...	...
384–1			
May 30	74	18	Constant
June 6	54	43	Decreasing
Aug. 2	68	...	...
Aug. 31	34	...	...
385–1			
May 30	21	12–13	Constant
June 6	65	9–11, 13	Constant
June 20	100	2	Increasing
386–1			
May 30	86	...	...
June 6	141	37–42, 44, 47–48, 50	Constant
Aug. 26	43	24, 26–27	Constant
Sept. 10	35	...	...
Oct. 15	17	...	...
387–1			
May 30	74	...	...
June 6	54	...	...
Aug. 2	57	...	...
Aug. 31	39	...	...
388–1			
May 30	127	...	...
June 6	55	12–15, 18	Decreasing
		42–43	Constant
		50–52, 56	Constant
Aug. 2	24	2–4	Constant
		24	Constant
Aug. 26	53	7	Constant
P. aurelia			
158*b*″			
May 15	48	34	Constant
May 19	54	...	...
July 17	40	...	...
Aug. 2	68	14	Constant
Aug. 31	27	...	...
250*b*″			
May 15	43	...	...
May 19	75	...	...
July 17	107	...	...
Aug. 26	33	8	Constant
Sept 28	20	6	Decreasing
360–2			
May 30	103	...	...
June 6	74	...	...
Aug. 31	47	...	...
Sept. 24	23	9–10	Constant
360–3			
May 30	98	...	...
June 6	32	...	...
Aug 2	53	...	...
Sept. 24	23	...	...
360–4			
May 30	54	16–18	Constant
June 6	118	8–10	Constant
Aug 24	46	...	...
364–1			
May 30	118	...	...
June 6	89	...	...
Sept. 10	30	...	...
Oct. 29	17	9	Constant
364–2			
May 30	56	12	Constant
June 6	76	5, 9	Constant
Aug. 2	77	11	Decreasing
Aug. 26	45	...	...
364–3			
May 30	88	...	...
June 6	118	10	Decreasing
July 26	15	4	Increasing
Sept. 24	28	...	...
Sept. 24	15	3	Increasing

Experiment 1 (table 2). Attempts to induce conjugation by a sudden decrease in the amount of food available.

Since several investigators have been able to induce conjugation by subjecting *Paramecium* from a flourishing culture to a sudden period of starvation, attempts were made to obtain conjugants by this method. The procedure employed was essentially that of Calkins and Cull (1907) and of Calkins and Gregory (1913). (*a*) Samples from two flourishing cultures of *P. caudatum* were transferred to Syracuse watch glasses. No conjugation appeared within three days, although, according to the foregoing writers, conjugants should appear within forty-eight hours if they appear at all. Perhaps the organisms were not 'mature.' Addition of distilled water after twelve hours did not induce a tendency to conjugate.

(*b*) Three clones, two of *P. caudatum* and one of *P. aurelia*, were employed. Beginning with the second day after transfer to fresh medium and continuing for the next ten days, samples from flourishing cultures were transferred every twenty-four hours to Syracuse watch glasses. The latter were examined for conjugants daily over a period of ten days. The results are given in table 2.

TABLE 2

ATTEMPTS TO INDUCE CONJUGATION BY SUDDEN DECREASE IN THE AMOUNT OF FOOD AVAILABLE

Race and date	Last date fresh media added	Procedure	Conjugation
		(Experiment 1*a*)	
P. caudatum 48–1 Mar. 2–Mar. 5, 1923........	Feb. 22	To watch glass; plus distilled water after 12 hours..................	None
P. caudatum 386–1 Mar. 2–Mar. 5, 1923 ..	Feb 12	To watch glass; plus distilled water after 12 hours.	None
		(Experiment 1*b*)	
P. caudatum 48–1 Mar. 8–Apr. 1, 1923	Mar. 6	Part to watch glass daily, and observed each day for ten days thereafter	None
P. caudatum 386–1 Mar. 8–Apr. 1, 1923	Mar. 6	Part to watch glass daily, and observed each day for ten days thereafter. ..	None
P. aurelia 364–1 Mar. 8–Apr. 1, 1923	Mar 6	Part to watch glass daily, and observed each day for ten days thereafter	None
P. aurelia 364–1 Mar. 8–Apr. 1, 1923	Mar. 6	Part to watch glass daily, and observed each day for ten days thereafter	Slight on March 10 in dishes started March 8 and March 9.

Race 48–1 was dormant from September 6, 1922, to January 12, 1923; after this date it was transferred to fresh food at monthly intervals up to the date of the experiment, March 8, 1923; the last transfer was on March 6. Races 386–1 and 364–1 (*P. aurelia*) had had no extended dormant period. No conjugants were observed with the races of *P. caudatum* (48–1, 386–1) either in the Syracuse dishes or in the milk-bottle culture used as a control. The same was true for one of the cultures of the race of *P. aurelia* (364–1). However, four days after starting another culture of the same race, a few conjugants appeared in watch glasses set out one or two days before. No other conjugants were seen, although transfers were continued for ten days more and observations for seventeen days.

Although the positive result in this one instance may indicate the value of a rapid decline from rich food conditions in the production of conjugation, the results were not sufficiently constant nor did the conjugants appear in large enough numbers to warrant the use of this method in any work demanding an extensive control of the process of conjugation.

Experiment 2 (table 3). Attempts to induce conjugation in mass cultures by the use of favorable salts.

A few preliminary experiments were carried out with mass cultures or populations of *Paramecium*, using $AlCl_3$ and $FeCl_3$, two of the most favorable salts employed by Zweibaum. These cultures had been dormant for several months, had been transferred to fresh food a month previously, and contained large numbers of *Paramecium* at the time of the experiment. Twenty cubic centimeters of the culture medium were added to equal volumes of N/4000 $AlCl_3$, N/8000 $FeCl_3$, and distilled water. This gave a concentration of N/8000 $AlCl_3$ and of N/16000 $FeCl_3$, strengths which, in Zweibaum's work, markedly favored conjugation. No conjugants, however, were observed in these experiments. These negative results are not necessarily opposed to Zweibaum's, however, since the animals had not been subjected to serial cultures. They show that appropriate concentrations of the favorable salts do not induce conjugation in themselves in the absence of the serial cultures. If Zweibaum's methods were applicable to all races, the use of populations rather than of clones would not alter the result. An experiment with a clone of *P. aurelia* (250), which had been subjected to one serial culture, also proved negative.

TABLE 3

ATTEMPTS TO INDUCE CONJUGATION IN MASS CULTURES BY DILUTION WITH SOLUTIONS OF $AlCl_3$, $FeCl_3$, AND WITH DISTILLED WATER

(Experiment 2)

Culture	Date	Last date fresh media added	Volume of media in cc.	Solution added	Volume of media in cc.	Conjugation
364	May 14, 1922	Apr. 15	20	Distilled water	20	None
364	May 14, 1922	Apr. 15	20	$AlCl_3$ N/4000	20	None
364	May 14, 1922	Apr. 15	20	$FeCl_3$ N/8000	20	None
363	May 14, 1922	Apr. 15	20	Distilled water	50	None*
363	May 14, 1922	Apr. 15	20	$AlCl_3$ N/4000	20	None*
363	May 14, 1922	Apr. 15	20	$FeCl_3$ N/8000	20	None*
250*b*″† *P. aurelia*	May 19, 1922	May 15	8 ⊙	Distilled water	5	None
250*b*″† *P. aurelia*	May 19, 1922	May 15	6 ⊙	$AlCl_3$ N/4000	20	None
250*b*″† *P. aurelia*	May 19, 1922	May 15	6 ⊙	$FeCl_3$ N/8000	20	None

* Dead May 15.

† Animals from a single clone (*P. aurelia*).

○ Diluted with 20cc. sterile bacterized media.

Experiment 3. Attempts to induce conjugation in clones by the use of favorable salts.

Beginning in September, 1923, a much more extensive series of experiments was begun, following Zweibaum's technique as closely as possible.

The following is a list of the races used, with a brief outline of their past histories. Daily observations were made only up to October 20, 1922; consequently, the recording of any case of conjugation after that date would be purely fortuitous.

PARAMECIUM AURELIA

Race 158 b″.—Isolated from a laboratory culture on May 1, 1922. No extensive conjugation epidemic observed; conjugants seen on June 18 and July 16; first used in conjugation tests October 20, 1923, from culture dormant since June 24 of the same year.

Race 364–1.—Isolated from a laboratory culture on May 19, 1922. A fairly large number of conjugants observed November 7, but no conjugants seen in daily observations for the next six days; an extensive epidemic of conjugation March 8 to March 12, 1923; several ex-conjugant lines started at this time. First employed in conjugation tests September 6, 1923; the following lines from different ex-conjugants were used: 108, 111, and 112, dormant since June 24, 1923; 116, dormant since May 18. Another series started from 364–1 on October 12, dormant since August 28.

PARAMECIUM CAUDATUM

Race 48–1.—Isolated May 19, 1922, from culture obtained from Strawberry Creek, April 23. Extensive epidemic of conjugation June 13 to 15; fair number of conjugants September 3 to 6; a few conjugants on December 10. First used in conjugation tests September 6, 1923, from culture dormant since May 18, 1923.

Race 48–2.—Isolated May 19, 1922, from culture obtained from Strawberry Creek, April 23. Extensive epidemic of conjugation June 13 to 19; a few conjugants August 1 to 4; September 9; May 18, 1923. First used in conjugation tests September 6, 1923, from culture dormant since June 24, 1923.

Race 360–5.—Isolated May 19, 1922, from laboratory culture. A few conjugants June 14 and 16, July 30, August 9 and 12, September 29 and 30. First used in conjugation tests October 20, 1923, from culture dormant since May 28, 1923.

Race 383–1.—Isolated May 19, 1922, from laboratory culture. Conjugation never observed. First used in conjugation tests September 6, 1923, from culture dormant since June 24, 1923.

Race 384–1.—Isolated May 19, 1922, from laboratory culture. Strong conjugation June 17, 1922; no other instances of conjugation observed. First used in conjugation tests September 6, 1923, from culture dormant since May 23, 1923.

Race 385–1.—Isolated May 19, 1922 (?), from laboratory culture (?). Strong epidemic of conjugation June 11 to 19; fair number of conjugants June 22; a few conjugants May 18, 1923; strong epidemic of conjugation May 28, 1923, to June 3, 1923. First used in conjugation tests September 6, 1923, from culture dormant since March 26, 1923.

Race 386–1.—Isolated May 19, 1922, from laboratory culture. A few conjugants July 13–18, 21, 23, 24, 26; September 19, 21, 22; December 26. First used in conjugation tests September 24, 1923, from culture dormant since August 2, 1923. Part of this culture had been transferred to fresh medium on September 9, and treated with sodium cyanide September 13 to 21.

Race 387–1.—Isolated May 19, 1922, from laboratory culture. Conjugation never observed. First used in conjugation tests September 24, 1923; dormant since September 2, 1923.

Race 390–1.—Isolated August 11, 1922, from pond at Pacific Grove, California. A few conjugants August 28–30; September 2–5, 1923. First used in conjugation tests September 24, 1923, from culture dormant since September 9, 1923. The animals in this culture had been treated with sodium cyanide, September 13 to 21.

At approximately five-day intervals from September 12 to November 3, cultures of these races were subjected to the same treatment as that employed by Zweibaum. Reference to table 5 shows that the results were largely negative except in race 385–1 (*P. caudatum*). In races 48–1, 48–2, and 384–1, although a few conjugants appeared with this treatment, they did not occur so regularly or in so great numbers as in the race used by Zweibaum or as in race 385–1 (table 6).

On the other hand, whenever *Paramecium* of race 385–1 was transferred from a serial culture to solutions of $AlCl_3$ N/10000 or of $FeCl_3$ N/15000–N/18000, conjugants always appeared in either one or the other salt solution, and, with only two exceptions, in both (tables 5 and 6). Although the relative number of conjugants was not so great as that found by Zweibaum, nevertheless the results taken by themselves would indicate the value of these salts in inducing conjugation under the conditions employed.

Beginning October 1, however, the animals were transferred not only from serial cultures to solutions of $AlCl_3$ and $FeCl_3$, but also to distilled water to which no salts had been added. All three sets of experimental cultures were carried along under parallel conditions; they were started at the same time and in the same size stender dishes. It was found that conjugation was occurring to approximately the same degree in all three sets of experiments (tables 4 to 6; fig. 1). It appeared, therefore, that members of race 385–1 would conjugate whenever they were transferred from a serial culture to one in which food conditions were poor, regardless of whether certain salts were present in the medium or not.

TABLE 4

COMPARISON OF NUMBER OF CONJUGATING CULTURES IN WHICH MEDIUM WAS DILUTED WITH $AlCl_3$, $FeCl_3$, OR WITH DISTILLED WATER; OR WITH MEDIUM UNDILUTED (SERIAL CULTURES). RATIO IN PER CENT OF NUMBER OF DISHES WITH CONJUGANTS TO TOTAL NUMBER OF DISHES, FOR SUCCESSIVE DAYS AFTER TRANSFER TO FRESH MEDIUM.

Days after transfer to fresh medium	$AlCl_3$ N/10000	$FeCl_3$ N/18000	Distilled water	No dilution
1				
2				
3				
4	8.3	4.3	4.0	11.1
5	4.2	8.7	8.0	16.7
6	56.5	59.1	52.0	55.6
7	43.5	50.0	44.0	41.1
8	45.0	68.4	47.8	30.8
9	16.7	23.5	28.6	39.2
10	26.7	35.4	26.3	30.8
11			12.5	14.2
12				14.2
13				14.2
Total number of dishes	24	23	25	18

On the other hand, among the animals carried past the fifth day in serial cultures and not transferred to other media, conjugants appeared at approximately the same time and with the same intensity as they did in dishes derived from these cultures and diluted with solutions of $AlCl_3$, $FeCl_3$, or with distilled water (tables 4–6; fig. 1). In other words, at this period of the experiment, animals belonging to race 385–1 conjugated, in practically every instance, within six days after being transferred to fresh medium; and exhibited this behavior regardless of whether they were transferred to dilute solutions of salts, to distilled water, or kept in the same dish of hay infusion without dilution.

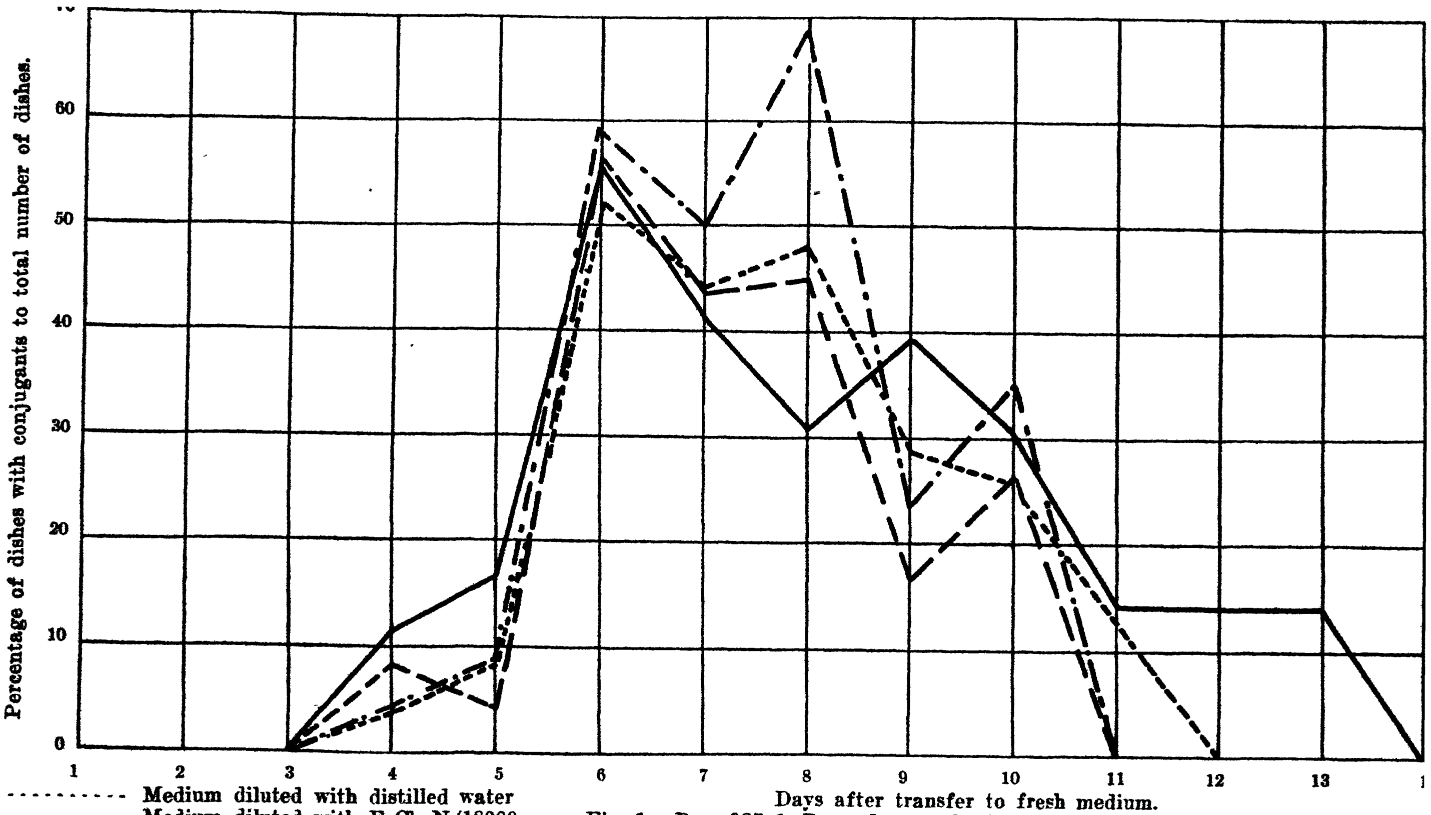

Fig. 1. Race 385–1, *P. caudatum*. Ratio in per cent of number of dishes with conjugants to total number of dishes, for successive days after transfer to fresh medium.

TABLE 5

Comparison of Instances of Conjugation in Race 385-1 with its Occurrence in Other Races

Race	Date of transfer to fresh medium																												
	Sept 14—Hay	Sept. 19—$AlCl_3$	Sept. 19—$FeCl_3$	Sept. 20—Hay	Sept. 24—Hay	Sept 24—$AlCl_3$	Sept. 24—$FeCl_3$	Oct 1—Hay	Oct 1—$AlCl_3$	Oct 1—$FeCl_3$	Oct 1—Distilled water	Oct 6—Hay	Oct. 6—$AlCl_3$	Oct 6—$FeCl_3$	Oct 6—Distilled water	Oct 6—Vitamin X (from hay)	Oct 11—Hay	Oct. 11—$AlCl_3$	Oct 11—$FeCl_3$	Oct. 11—Distilled water	Oct. 11—Vitamin X (from hay)	Oct. 16—Hay	Oct. 16—$AlCl_3$	Oct. 16—$FeCl_3$	Oct. 16—Distilled water	Oct 16—Vitamin X	Oct. 20—Hay	Oct 20—$AlCl_3$	Oct 20—$FeCl_3$
P. aurelia																													
158*b*″																											0		.
364-1	0			0	0	0		0	0	0		0	0	0	0		0				0	0				0	0		.
P. caudatum																													
48-1	0	0	0	0	0	0	0	0	X	X	X	0	0	0	0		0	0	X	X		0	X	X	X	0	0	0	0
48-2	X	0	0	0	0	0	0	0	0	0	0	0		0			0	X	X	X		0	0	X	X		0		
360-5																											0		
383-1	0			0	0			0				0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0
384-1	X	0	0	0	0	0	X	X	0	0		X	X	X	0		0	X	X	X		0	0	0	X	0	0		0
386-1						0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0		
387-1					0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0		0		0	0	0	0
390-1						0		0	0	0		0		0			0	0	0	0		0	0	0	0		0		
385-1	X	X	X	0	0	X	X	0	X	0		X	X	X	X	X	X	X	X	X	X	0	X	X	X	0	X	X	X

Race	Date of transfer to fresh medium																											
	Oct. 20—Distilled water	Oct. 20—Vitamin X	Oct. 25—Hay	Oct. 25—$AlCl_3$	Oct 25—$FeCl_3$	Oct. 25—Distilled water	Oct 27—Hay	Oct. 30—Hay	Oct. 30—$AlCl_3$	Oct. 30—$FeCl_3$	Oct 30—Distilled water	Nov. 3—Hay	Nov. 3—Distilled water	Nov 8—Hay	Nov 15—Hay	Nov. 22—Hay	Nov 30—Hay	Dec 8—Hay	Jan 10—Hay	Jan 17—Hay	Jan. 24—Hay	Jan. 28—Hay	Jan. 31—Hay	Feb 5—Hay	Feb 14—Hay	Feb. 15—Hay	Mar. 4—Hay	June 13—Hay
P. aurelia																												
158*b*″			0	0		0		0				0	0	0	0	0			0									0
364-1			0	0	0	0		X	0	X	X	0	0	X	X	X	X	0	0		0		X	0			0	0
P. caudatum																												
48-1	0	0	0	0	0	0		0	0	0	0	0	0	0	X	X	X	0	0									
48-2			0					0				0	0	0	X	X	0		0									0
360-5			0					0				0		0	0	0	X		0									
383-1	0	0	0	0	0	0		0			0	0	0	0	0	0												X
384-1		0	0	X	X	0		0	X			0	0	0	0	0			0									
386-1		0	0	0	0	0		0				0	0	X	0	0			0									0
387-1	0	0						X	0	0	0	0	0	X	0	0	0	0	X		0		0	0			X	0
390-1		0						0				0		0	0	0												
385-1	X	0	X	X	X	X	X	X	0	X	X	X	X	X	X	X	X	X	X	0	X	X	X	X	X	X	X	X

[X = conjugation. 0 = no conjugation]

TABLE 6

COMPARISON OF MAXIMUM INTENSITY OF CONJUGATION IN RACE 385–1 WITH ITS MAXIMUM INTENSITY IN OTHER RACES

Race	Date of transfer to fresh medium																												
	Sept 14—Hay	Sept. 19—$AlCl_3$	Sept 19—$FeCl_3$	Sept. 20—Hay	Sept. 24—Hay	Sept. 24—$AlCl_3$	Sept 24—$FeCl_3$	Oct. 1—Hay	Oct. 1—$AlCl_3$	Oct. 1—$FeCl_3$	Oct. 1—Distilled water	Oct 6—Hay	Oct. 6—$AlCl_3$	Oct. 6—$FeCl_3$	Oct 6—Distilled water	Oct 6—Vitamin X (from hay)	Oct. 11—Hay	Oct. 11—$AlCl_3$	Oct. 11—$FeCl_3$	Oct. 11—Distilled water	Oct. 11—Vitamin X (from hay)	Oct 16—Hay	Oct. 16—$AlCl_3$	Oct 16—$FeCl_3$	Oct. 16—Distilled water	Oct 16—Vitamin X	Oct 20—Hay	Oct 20—$AlCl_3$	Oct. 20—$FeCl_3$
P. aurelia																													
158*b*″																											–		
364–1	–			–	–	–		–	–	–		–	–	–	–		–				–	–				–	–		
P. caudatum																													
48–1	–	–	–	–	–	–	–	–	1	1	1	–	–	–	–		–	–	1	1		–	1	1	2	–	–	–	–
48–2	2	–	–	–	–	–	–	–	–	–	–	–		–			–	1	1	1		–	–	1	1		–		
360–5																											–		
383–1	–			–	–			–				–	–	–	–	–	–	–		–	–	–	–		–	–	–	–	–
384–1	1	–	–	–	–	–	1	1	–	–		1	1	1	–		–	2	2	1		–	–	–	1	–	–		–
386–1						–		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		–	–		
387–1					–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–		–		–	–	–	–
390–1						–		–	–	–		–		–			–	–	–	–		–	–	–	–		–		
385–1	2	1	1	–	–	2	2	–	2	–		3	2	2	2	3	2	3	2	3	4	–	1	2	3	–	2	1	3

Race	Date of transfer to fresh medium																											
	Oct 20—Distilled water	Oct. 20—Vitamin X	Oct 25—Hay	Oct. 25—$AlCl_3$	Oct 25—$FeCl_3$	Oct 25—Distilled water	Oct. 27—Hay	Oct 30—Hay	Oct. 30—$AlCl_3$	Oct. 30—$FeCl_3$	Oct. 30—Distilled water	Nov 3—Hay	Nov. 3—Distilled water	Nov. 8—Hay	Nov 15—Hay	Nov. 22—Hay	Nov 30—Hay	Dec. 8—Hay	Jan. 10—Hay	Jan. 17—Hay	Jan. 24—Hay	Jan. 28—Hay	Jan. 31—Hay	Feb. 5—Hay	Feb. 14—Hay	Feb. 15—Hay	Mar. 4—Hay	June 13—Hay
P. aurelia																												
158*b*″			–	–		–		–				–	–	–	–	–			–									–
364–1			–	–	–	–		1	–	1	1	–	–	1	1	1	1	–	–		–		1	–			–	–
P. caudatum																												
48–1	–	–	–	–	–	–		–	–	–	–	–	–	–	1	1	1	–	–									
48–2			–					–				–	–	–	1	1	–		–									–
360–5			–					–				–		–	–	–	1		–									
383–1	–	–	–	–	–	–		–			–	–	–	–	–	–												1
384–1		–	–	1	1	–		–	1				–	–	–	–			–									
386–1		–	–	–	–	–		–				–	–	1	–	–			–									–
387–1	–	–						1	–	–	–	–	–	1	–	–	–	–	1		–		–	–			1	–
390–1		–						–				–		–	–	–												
385–1	2	–	2	2	3	3	3	3	–	2	1	4	2	3	4	3	2	1	2	–	2	4	3	3	2	1	3	2

[1 = conjugation 5% or less. 2 = conjugation 6–20%. 3 = conjugation 21–50%. 4 = conjugation more than 50%. – = no conjugation.]

Experiment 4. Further experimental work on the behavior of race 385–1.

When it was seen that conjugation was appearing with equal facility in the serial cultures and in those transferred to solutions of salts or to distilled water, the latter sets of experiments were discontinued, and conjugation was induced merely by transferring the ciliates in serial culture from one dish of hay infusion to a fresh culture of the same medium. In such cultures, conjugants usually appeared within six days from the time of transfer, were present for three or four days, and ordinarily did not reappear in the same dish of culture medium, unless fresh food was added. The degree to which conjugation occurred with this treatment and the extent of the experiments are indicated in tables 5–10. These tables present in a condensed fashion a summary of the experiments performed with this race, and should be considered in connection with the following detailed account.

History.—The culture was started in a crystallizing dish on September, 6, 1923, from animals which had been transferred to fresh infusion in a quarter-pint milk bottle on March 26, 1923, and had not received additional food in the intervening period of 164 days. The dish was first observed for conjugants on September 12; no conjugation had occurred by September 14, when the animals were transferred to fresh medium.

Serial culture begun September 14 showed conjugants on the 18th to 20th; subcultures to $AlCl_3$ and $FeCl_3$, started on the 19th and running to the 24th, both had conjugants on the 20th.

Fresh serial culture on the 20th gave no conjugants by the 24th, when it was discontinued and a new serial culture was started; but subcultures on $AlCl_3$ and $FeCl_3$, begun on this date and running to October 1, gave conjugants; the former on September 25th and 26th; the latter on the 25th, 26th, and 28th.

Serial culture started on the 24th; some rice grains were added on the 27th; died out on October 1, evidently due to too heavy bacterization.

Serial culture started October 1 from subculture on $AlCl_3$ of September 24 gave no conjugants up to the time it was discontinued on October 6. Conjugants appeared in subculture on $AlCl_3$ on the 4th; none appeared in subculture on $FeCl_3$. Subcultures running from

October 6 to 11 gave conjugants on $AlCl_3$, October 7 and 8; $FeCl_3$, October 7–10; distilled water, October 7 and 8; vitamin X (see below), October 7–9.

Serial culture started October 6 had conjugants on the 11th; a branch culture, started on the latter date without dilution, gave conjugants on the 12th and 13th; subcultured on the 20th to $AlCl_3$, $FeCl_3$, and distilled water, gave no conjugants. Subcultures made on the 11th with $FeCl_3$ and distilled water had conjugants on the 12th to 14th; with $AlCl_3$, on the 12th and 13th; with vitamin X, on the 12th to 16th. A continuation of the vitamin X culture to October 30, with subcultures on the 17th to 20th with $AlCl_3$, $FeCl_3$, and distilled water, produced no conjugants.

Serial culture of October 11 gave conjugants on the 12th and on the 16th to 19th; subcultures of October 17 gave conjugants with $AlCl_3$, on the 17th and 19th; with $FeCl_3$, on the 17th and 18th; with distilled water, on the 17th, 18th, and 19th. Subcultures of October 20 gave conjugants on the 20th with $AlCl_3$ and $FeCl_3$; none with distilled water.

Serial culture of October 17 gave no conjugants up to the time it was discontinued on the 20th; subcultures of the latter date had conjugants with $AlCl_3$ on the 21st; with $FeCl_3$, on the 21st and 22d; with distilled water, on the 21st, 22d, and 23d. Serial culture of October 17 with vitamin X gave no conjugants; nor did three subcultures made on the 20th.

Serial culture of October 20 gave conjugants on the 25th to 28th. On the 25th, 8 subcultures were made with $AlCl_3$; 8 with $FeCl_3$; and 9 with distilled water; all conjugating on the 26th to 28th; some dishes in each group had conjugants on the 29th and 30th. Serial culture of October 20 with vitamin X were negative as in the preceding series; also subcultures made on the 25th. No conjugation resulted with serial culture of October 20 from old medium of October 6 nor with subculture with distilled water made on the 25th.

The general behavior of the races has been indicated sufficiently by the foregoing detailed account, therefore only a skeleton outline will be given of the subsequent work.

October 25. Serial culture *a*—no conjugants; serial culture *b* and two branch cultures made October 31 and November 3—both with conjugants. Subcultures with $FeCl_3$ and distilled water—conjugants on October 31; with $AlCl_3$—no conjugants. Subculture on November 3 with distilled water—conjugants. No conjugation with vitamin X; *Paramecium* on vitamin X returned to hay infusion on October 30.

October 27. Serial cultures *a*, *b*, and *c*—all with conjugants. No conjugants on subcultures with $AlCl_3$, $FeCl_3$, and distilled water.

October 31. Serial culture *a*—conjugants; subculture with distilled water, November 3—no conjugants. Serial culture *b* and subculture with distilled water, November 3—both with conjugants. Serial cultures *c* and *d*—both with conjugants; none in subcultures with distilled water, November 3; serial cultures *e* and *f* and subcultures with distilled water, November 3—all with conjugants. Animals from vitamin X, transferred to hay infusion on October 30, conjugation November 2–3.

November 3. Experiments with $AlCl_3$ and $FeCl_3$ discontinued. 9 serial cultures —conjugation in 7.

November 8. Experiments with distilled water discontinued. 15 serial cultures —conjugation in 7.

November 15. 21 serial cultures—conjugation in 15.

November 22. 17 serial cultures—conjugation in 10.

November 30. 16 serial cultures—conjugation in 10. Fifteen of these cultures were observed daily up to January 10, without change of medium—a long, dormant period.

December 8. 4 serial cultures—conjugation in 2. These cultures were without fresh medium to January 10.

January 10, 1924. 21 serial cultures—conjugation in 11. The distribution of the conjugating cultures according to their dormant periods was: 1–75 days; 1–68 days; 3–49 days; 5–41 days; 2–33 days.

January 17. 9 serial cultures—conjugation in none.

January 24. 7 serial cultures—conjugation in 4.

January 31. 11 serial cultures—conjugation in 9.

February 5. 3 serial cultures—conjugation in 3.

February 14. 3 serial cultures—conjugation in 3.

February 15. 4 serial cultures—conjugation in 2.

March 4. 8 serial cultures—conjugation in 6.

April 26. 4 serial cultures—no observations.

June 13. 18 serial cultures (some dormant since March 4)—conjugation in 11.

The foregoing is an account of the number of dishes started on each date, with the number showing conjugants; it does not include the ex-conjugant lines. Table 7 gives the number of dishes started on fresh hay infusion on the various dates, together with the number of dishes derived from that series. It shows the total number of dishes for every series on each successive day until all the dishes of the series were discontinued. Table 8 shows the number of dishes with conjugants for each day after transfer to fresh medium; and table 9 is a record, for each series of transfers, of the percentage of dishes having conjugants on any particular day to the total number of culture dishes being observed on that day.

TABLE 7

Record of Total Number of Culture Dishes on Successive Days after Transfer to Fresh Medium

Race 385–1, *P. caudatum*

Date of transfer to fresh medium	Number of cultures started	Total number of culture dishes on successive days after transfer to fresh medium																																										
		Days after transfer to fresh medium																																										
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	
Sept 14, 1923	1	1	1	1	1	1	3	2	2	2	2	0																																
Sept. 20, 1923	1	1	1	1	1	2	2	2	2	2	2	2	1	1	1	1	1	0																										
Oct. 1, 1923	2	2	2	2	2	2	4	4	4	4	4	0																																
Oct. 6, 1923	1	1	1	1	1	1	5	5	5	5	5	5	4	4	4	4	4	4	4	4	1	1	1	1	1	1	1	1	0															
Oct. 11, 1923	1	1	1	1	1	1	4	4	4	4	3	3	3	3	0																													
Oct. 17, 1923	2	2	2	2	6	6	6	6	6	0																																		
Oct. 20, 1923	4	4	4	4	4	4	36	36	36	36	36	11	5	1	1	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0–84 days
Oct. 25, 1923	2	2	2	2	2	2	5	5	5	5	3	3	3	3	3	3	3	3	3	3	3	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0–46 days
Oct. 27, 1923	3	3	3	3	11	11	11	11	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1–64 days 0–79 days
Oct. 30, 1923	4	4	4	4	4	9	9	9	9	9	9	9	9	9	9	9	9	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	3	3	3	3	3	3	3	0–73 days
Nov. 3, 1923	10	10	10	10	10	10	11	11	11	11	11	11	11	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	7	7	7	7	7	7	7	7	5	5	5	5	5	5	5	4–44 days 0–69 days
Nov. 8, 1923	15	15	15	15	15	15	15	15	10	10	10	10	10	10	10	5	5	5	5	5	5	5	5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0					
Nov. 15, 1923	21	21	21	21	21	21	21	21	11	11	11	11	11	11	11	11	4	4	4	4	4	4	4	4	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0–57 days
Nov. 22, 1923	17	17	17	17	17	17	17	17	17	5	5	5	5	5	5	5	5	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0–51 days
Nov. 30, 1923	16	16	16	16	16	16	16	16	16	16	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	4–43 days 0–62 days
Dec. 8, 1923	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0				
Jan. 10, 1924	21	21	21	21	21	20	20	20	20	5	5	5	5	5	5	3	3	3	3	3	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0–57 days
Jan. 17, 1924	9	9	9	9	9	9	9	9	4	4	4	4	3	3	3	0																												
Jan. 24, 1924	7	7	7	7	7	6	6	6	0																																			
Jan. 28, 1924	4	4	4	4	4	4	4	4	4	0																																		
Jan. 31, 1924	11	11	11	11	11	11	11	10	10	10	10	10	10	10	7	3	3	3	3	3	3	3	3	3	3	3	1	1	1	1	1	1	1	1	0									
Feb. 5, 1924	3	3	3	3	3	3	3	3	5	5	4	3	3	3	3	3	3	3	3	1	1	1	1	1	1	1	1	1	1	0														
Feb. 14, 1924	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0																							
Feb. 15, 1924	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	3	3	3	3	0																								
Mar. 4, 1924	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	0															
June 13, 1924	18	18	18	18	18	16	13	12	12	12	12	12	0																															

TABLE 8

Record of Number of Dishes Having Conjugants on Successive Days after Transfer to Fresh Medium

Race 385–1, *P. caudatum*

Date of transfer to fresh medium	Number of dishes with conjugants on successive days after transfer to fresh medium																																									
	Days after transfer to fresh medium																																									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Sept. 14, 1923				1	1	3																																				
Sept. 20, 1923					2	2		1																																		
Oct. 1, 1923			1			4	4	2	1																																	
Oct. 6, 1923					1	5	5	3	1	1																																
Oct. 11, 1923	1				1	4	3	3		2																																
Oct. 17, 1923				3	2	1																																				
Oct. 20, 1923	1	1			2	27	27	27	11	10	1				1	1			1																							
Oct. 25, 1923						3	2	4	3	3	3																															
Oct. 27, 1923			3	5	2	2	2	2	2	2	2	2	1																										1			
Oct. 30, 1923		3	4	3	2	3	2	1	1																			1	1													1
Nov. 3, 1923	1			1	4	5	4	3	4	3	3	3	2	2							1																					
Nov. 8, 1923	1	2	3	2	2	2	2	2	2	3	4	4	3	3	1																											
Nov. 15, 1923		1	3	4	5	6	9	5	5	4	4	1	1	1									1																			
Nov. 22, 1923	1	1	2	3	8	5	5	7	3	3	3	2	1	1	1	1			1	1																						
Nov. 30, 1923			3	6	2	3	4	5	3	3	2	3	1	2	1																											
Dec. 8, 1923			1	1	1	1		1		1																																
Jan. 10, 1924		2	9	7	2	2																																				
Jan. 17, 1924																																										
Jan. 24, 1924	1		1	3	3	2																																				
Jan. 28, 1924			1	1	1	1																																				
Jan. 31, 1924			2	4	4	3	3	3	4	2	5	6	5	3	3	2	1	2	2	1	1	1	1		1																	
Feb. 5, 1924		1		1		1	1	1	1	1	1																															
Feb. 14, 1924					1	1		1	1		1	1	1	2	2		1		1																							
Feb. 15, 1924					1	1	1	1						1		1		1																								
Mar. 4, 1924	2	2	2	2	2	3	3	2	2	3	1	2	2	2	3	2	2	2	3	3	2	1	1	1	1																	
June 13, 1924		2	1	3	1	1	1				2																															

TABLE 9

Ratio in Per Cent of Number of Dishes with Conjugants to Total Number of Dishes on Successive Days after Transfer to Fresh Medium

Race 385–1, *P. caudatum*

Date of transfer to fresh medium	Days after transfer to fresh medium																																									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Sept 14, 1923				100	100	100																																				
Sept. 20, 1923					100	100		50																																		
Oct. 1, 1923			50			100	100	50	25																																	
Oct. 6, 1923					100	100	100	60	20	20																																
Oct. 11, 1923	100				100	100	75	75		67																																
Oct. 17, 1923				50	33	17																																				
Oct. 20, 1923	25	25			50	75	75	75	31	28	9				50	50			50																							
Oct. 25, 1923						60	40	80	60	100	100																															
Oct. 27, 1923			100	45	18	18	18	29	29	29	29	29	14																										33			
Oct. 30, 1923		75	100	75	22	33	22	11	11																			25	25													33
Nov. 3, 1923	10			10	40	45	36	27	36	27	27	27	25	25							13																					
Nov. 8, 1923	7	13	20	13	13	13	13	20	20	30	40	40	30	30	20																											
Nov. 15, 1923		5	14	19	24	28	42	45	45	36	36	9	9	9									25																			
Nov. 22, 1923	6	6	12	17	46	29	29	41	60	60	60	40	20	20	20	20			33	33																						
Nov. 30, 1923			18	37	12	18	25	31	18	20	13	20	7	13	7																											
Dec. 8, 1923			33	33	33	33		33		33																																
Jan. 10, 1924		9	42	33	10	10																																				
Jan. 17, 1924																																										
Jan. 24, 1924	14		14	43	50	33																																				
Jan. 28, 1924			25	25	25	25																																				
Jan. 31, 1924			18	36	36	27	30	30	40	20	50	60	50	43	100	67	33	67	67	33	33	33	33		33																	
Feb. 5, 1924		33		33		33	33	20	20	25	33																															
Feb. 14, 1924					33	33		33	33		33	33	33	67	67		33		33																							
Feb. 15, 1924					25	25	25	25						25		25		25																								
March 4, 1924	25	25	25	25	25	38	38	25	25	38	13	25	25	25	38	25	25	25	38	38	25	13	13	13	18																	
June 13, 1924		11	6	17	7	8	8				17																															

TABLE 10

Record of Maximum Intensity of Conjugation on Successive Days after Transfer to Fresh Medium

Race 385-1, *P. caudatum*

[1 = conjugation 5% or less. 2 = conjugation 6-20%. 3 = conjugation 21-50% 4 = conjugation more than 50%.]

Date of transfer to fresh medium	Days after transfer to fresh medium																																									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Sept. 14, 1923				1	2	1																																				
Sept. 20, 1923					2	2		1																																		
Oct. 1, 1923			2			3	3	1	1																																	
Oct. 6, 1923					3	3	4	2	1	1																																
Oct. 11, 1923	1				1	2	3	1		1																																
Oct. 17, 1923				3	1	1																																				
Oct. 20, 1923	1	1			1	3	3	2	2	1	1				1	1			1																							
Oct. 25, 1923						2	1	1	2	3	3																															
Oct. 27, 1923			2	2	1	2	2	2	3	1	2	1	1																										1			
Oct. 30, 1923		2	3	2	1	3	3	1	1																			1	1													1
Nov. 3, 1923	1			1	2	4	2	1	1	2	4	4	2	1							1																					
Nov. 8, 1923	1	3	1	1	1	1	1	1	1	1	3	2	2	2	1																											
Nov. 15, 1923		1	1	3	2	2	4	4	2	2	1	1	1	1									1																			
Nov. 22, 1923	1	1	2	1	1	2	3	2	1	2	1	1	1	1	1	1			1	1																						
Nov. 30, 1923			1	1	1	1	1	1	1	1	2	1	1	1	1																											
Dec. 8, 1923			1	1	1	1		1		1																																
Jan. 10, 1924		1	2	2	1	1																																				
Jan. 17, 1924																																										
Jan. 24, 1924	1		2	1	1	1																																				
Jan. 28, 1924			4	3	1	1																																				
Jan. 31, 1924			1	1	2	1	2	1	2	1	2	2	2	2	2	3	2	2	2	3	2	2	1		1																	
Feb. 5, 1924		1		1		1	1	1	3	2	1																															
Feb. 14, 1924					1	1		1	1		1	1	1	1	2		1		2																							
Feb. 15, 1924					1	1	1	1						1		1		1																								
Mar. 4, 1924	2	2	2	2	2	2	3	2	2	1	1	1	1	2	2	3	2	3	2	2	1	2	2	1	1																	
June 13, 1924		1	1	1	1	2	2				1																															

Parallel series were carried for other races, and served as controls. The comparative behavior of race 385–1 and the other lines is indicated in table 5, which gives a record of any instance of conjugation in any of the transfers made. Table 6 gives the maximum intensity of conjugation at each transfer for the different races; table 10 shows the distribution of conjugation intensity for race 385–1 on successive days after transfer to fresh medium.

It may be seen from the tables and from the detailed account given above that conjugation was occurring fairly extensively in race 385–1. Permanent preparations made from time to time and stained with borax carmine, showed that the unions observed were actual conjugations and not merely a superficial adhesion of the protoplasm of the two animals. The behavior of the conjugants was normal in every way, with no indication of any irregularity in the process. In some of the stronger epidemics of conjugation, temporary unions of three individuals were occasionally observed; and one case was seen in which four animals were stuck together. Instances of like nature have been described by other observers.

The length of time during which pairs of conjugants continued to occur in a culture varied from one day to thirteen successive days. In a few instances, conjugants were observed at intervals, without any addition of fresh medium to the culture, for as long as three weeks. Strong epidemics of conjugation usually did not last more than three or four days, although a few pairs of conjugants might still be seen several days after the main portion of the epidemic was over. Instances of conjugation as late as twenty days after transfer to fresh food were very rare; the most extreme case of conjugation occurred in a culture dormant for forty-two days (table 8). There seemed to be a slight tendency for a few conjugants to reappear in old cultures in which none had been seen for some little time previously. This may indicate a tendency for conjugation to appear in cycles, although the possibility of again obtaining conjugants from cultures in which conjugation had been seen only five days before does not point to the occurrence of any conjugation-cycle in this race. By making transfers at appropriate intervals, it was possible to obtain a series that gave conjugants practically every day (table 8).

In conjugating cultures of *Paramecium,* the animals were frequently aggregated in groups. We may refer to this behavior as clumping. The phenomenon was early described by Balbiani (1861), who believed it to be peculiar to periods of conjugation. The animals collect in little whitish clumps around objects floating in the water or along the sides of the dish, or around particles at the bottom of the culture. It is possible that the process of conjugation may be analogous to the phenomenon of agglutination (Doflein, 1907).

In the course of the observations made on the cultures in stender dishes, aggregations of *Paramecium* were frequently observed in cultures recently transferred to fresh medium. Practically all the ciliates were collected into one or a few clumps or else restricted to a narrow region around the edge of the dish. Any conjugants occurring in these dishes were limited usually to one or two of the clumps; and practically none were found in other parts of the dish. The results of a rather careful recording of the occurrence of such clumps and the presence of conjugants in the same dish are summarized in table 11. Fifty cultures in which conjugation occurred were taken at random and compared with fifty cultures in which conjugation did not occur under like conditions, the presence or absence of clumps being noted in each case.

TABLE 11

COMPARISON OF THE OCCURRENCE OF CLUMPING IN 50 CONJUGATING AND IN 50 NON-CONJUGATING CULTURES

	Conjugation		No conjugation	
	Clumping	No clumping	Clumping	No clumping
Number of cultures....	32	18	29	21

Briefly, the conclusions are that clumping frequently occurs in cultures transferred to fresh medium or to distilled water or to weak solutions of salts, and that it is of two kinds: one around masses of bacterial zooglea, the other around the margin of the dish. Since the animals give the avoiding reaction on passing out of the clump, the presence of CO_2 evidently aids in maintaining and in enlarging the clump after it is first formed. Clumps around masses of zooglea are formed primarily to obtain food. Clumps around the margin of the dish are thigmotactic in origin; in one instance, a very dense clump formed repeatedly under a small glass chip lying in the center of the dish; this collection was not due to mechanical trapping. The fact that the members of a clump are in constant motion is opposed to the view that this is an agglutination phenomenon. Cultures recently transferred to fresh medium or to distilled water are apt to develop conjugants. But clumping is not diagnostic of conjugation, since it occurs with equal frequency in cultures of this type regardless of whether or not conjugation ensues (cf. Maupas, 1889).

Time of occurrence of conjugation.—In the first portion of the experiment, conjugation appeared regularly about the sixth day after transferring the animals to new medium (fig. 2*a*). The great majority of conjugations occurred from the fourth to the ninth day after the addition of fresh food. As the experiment progressed, however, this periodicity in occurrence became less marked; there was no distinct tendency for conjugation to occur more frequently on the sixth day than on the days immediately preceding or following, but it appeared with more or less equal frequency from the third to the eleventh day (fig. 2*b*). During the last portion of the experiment, this flattening of the curve of conjugation was even more marked (fig. 2*c*); the extent of conjugation was practically unchanged from the fourth to the nineteenth day, with a fair percentage of conjugants as late as the twenty-third day after transfer to fresh food.

Taking the experiments as a whole, however, the highest percentage of conjugations occurs from the sixth to the eighth day (fig. 2*d*).

Behavior of ex-conjugants.—Single conjugating pairs were placed in depression slides, the ex-conjugants isolated after separation, and the individuals from the first two divisions of each ex-conjugant again isolated and used to start separate clones. As is frequently the case with *Paramecium* under these conditions, a high mortality occurred among the ex-conjugants. The conjugating behavior of those clones derived from ex-conjugants which successfully reorganized is given in table 12. There was considerable difference in the behavior of the different ex-conjugants in ability to conjugate, a fact already pointed out by Calkins and Gregory (1913). Some lines, such as j′–1–2, g–2, and f′–2–1 conjugated under the same conditions as did the main culture, 385–1. In other ex-conjugants, however, conjugation appeared to have weakened or destroyed their conjugating power, at least so far as their response to a certain set of conditions was concerned. Such were lines d–2–1 and i–1–1. Line f–1–1 retained the power to conjugate for about six weeks after reorganization; lost it for the next three months; but had regained it when tested three months later. In ex-conjugant race b–1–1, an F_2 generation, no conjugation could be induced.

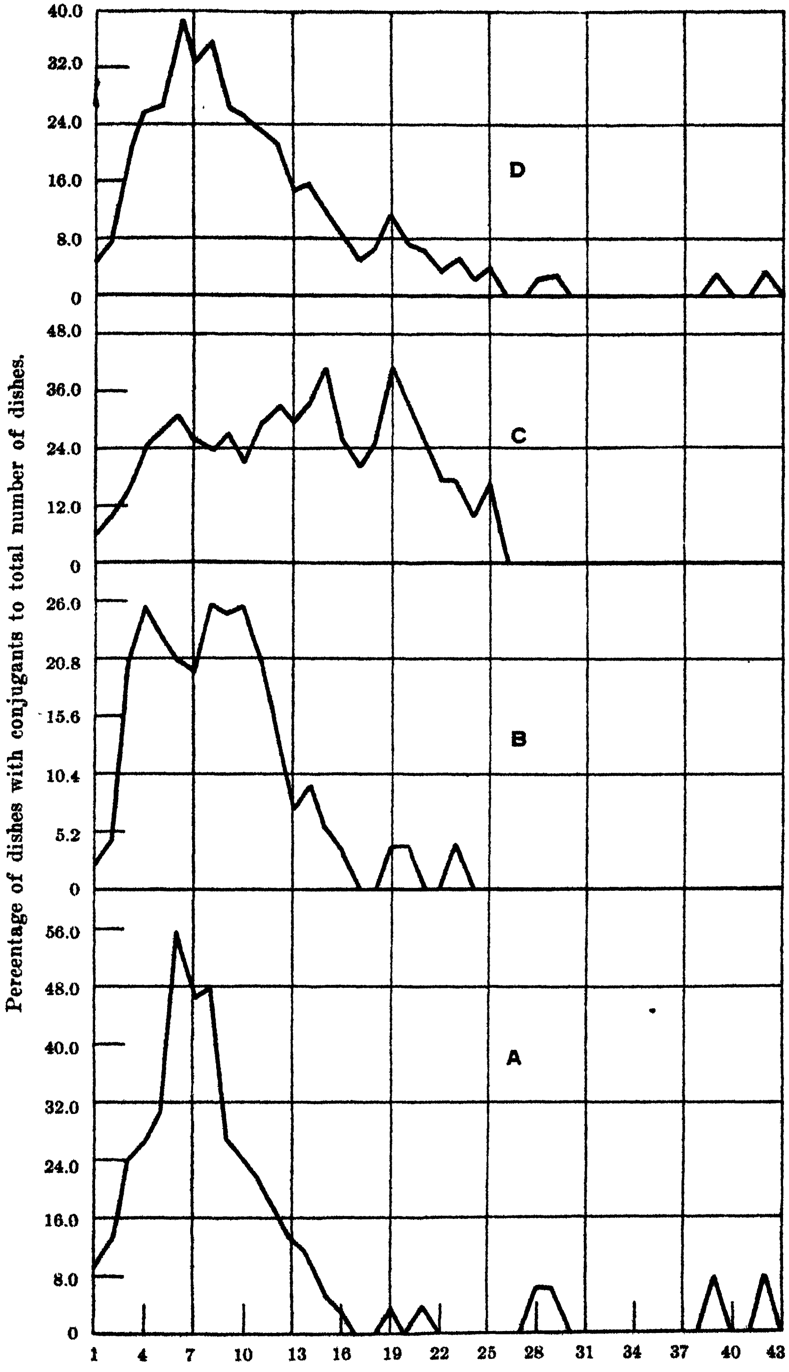

Fig. 2. Race 385–1, *P. caudatum*. Percentage of dishes with conjugants to total number of culture dishes, for successive days after transfer to fresh medium.

A—Sept. 14, 1923, to Nov. 8, 1923. C—Jan. 28, 1924, to Mar. 31, 1924.
B—Nov. 15, 1923, to Jan. 25, 1924. D—Sept. 14, 1923, to Mar. 31, 1924.

TABLE 12

COMPARISON OF THE TENDENCY TO CONJUGATE AMONG DIFFERENT EX-CONJUGANTS OF RACE 385–1

[X = conjugation. 0 = culture discarded. Numbers in parenthesis represent number of culture dishes.]

Ex-conj. line and date of transfer to fresh medium	Number of cultures started	Days after transfer to fresh medium																												
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
f'-1-2:																														
Oct. 31, 1923	1											X	X	X	X															0–47 days
Nov. 8, 1923	1																							0						
Nov. 15, 1923	1			X	X	X	X	0																						
Nov. 22, 1923	1						X	X	X	0																				
Nov. 30, 1923	1		X									X	X																	0–42 days
Jan. 10, 1924	1		X	X					0																					
d-2-1:																														
Oct. 31, 1923	1		X	X	(2)					(1)																				0–47 days
Nov. 3, 1923	1			(2)																	(1)								0	
Nov. 8, 1923	1								0																					
Nov. 15, 1923	1								0																					
Nov. 22, 1923	1																													0–51 days
Nov. 30, 1923	1															0														
f-1-1:																														
Oct. 31, 1923	1			X	(3) 3 X	X X	(4)			(3)						(2)							(1)							0–30 days
Nov. 3, 1923	1					X	0																							
Nov. 5, 1923	1													X		X	X	X	X	X				X			X			0–52 days
Nov. 8, 1923	1								0																					
Nov. 15, 1923	1						X	X	0																					
Nov. 22, 1923	1									0																				
Nov. 30, 1923	1									0																				
Dec. 8, 1923	1												X																	0–34 days
Jan. 10, 1924	2									(1)								0												
Jan. 18, 1924	1							0																						
Jan. 24, 1924	1								0																					
Jan. 31, 1924	1															0														
Feb. 14, 1924	1																				0									
Mar. 4, 1924	1																													0–29 days
June 13, 1924	1					X	X	X	X			0																		

TABLE 12—(*Concluded*)

[X = conjugation. 0 = culture discarded. Numbers in parenthesis represent number of culture dishes.]

Ex-conj. line and date of transfer to fresh medium	Number of cultures started	Days after transfer to fresh medium																												
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
g-2:																														
Oct. 31, 1923	1																				X			(2)		X	X	X		0–39 days
Nov. 8, 1923	1								0																					
Nov. 15, 1923	1								0																					
Nov. 22, 1923	1									0																				
Nov. 30, 1923	2			X	X																									0–42 days
Jan. 10, 1924	1		X	X												0														
Jan. 24, 1924	1					X			0																					
Jan. 31, 1924	1								X	X						0														
Feb. 14, 1924	1								X									X			0									
Mar. 4, 1924	1		X	X	X	X																								0–28 days
f′-2-1:																														
Oct. 31, 1923	1				(2)					(1)																				0–72 days
Nov. 3, 1923	1																				0									
Nov. 8, 1923	1					X	X	X	X	X	X	X	X	X	X	X	X	X					0							
Nov. 15, 1923	1								0																					
Nov. 22, 1923	2		X	X		X				0																				
Nov. 30, 1923	1																													0–42 days
Jan. 10, 1924	1			X					0																					
i-1-1:																														
Oct. 31, 1923	1				(2)					(1)																				0–31 days
Nov. 8, 1923	1																							0						
Nov. 15, 1923	1								0																					
Nov. 22, 1923	1																													0–50 days
Nov. 30, 1923	2			X																										0–42 days
Dec. 8, 1923	1																													0–34 days
Jan. 10, 1924	2								(1)	0																				
b-1-1 (ex-conj. of f-1-1):																														
Nov. 24, 1923	1							0																						
Nov. 30, 1923	1									0																				
Dec. 8, 1923	1																													0–34 days
Jan. 10, 1924	1										0																			

Because of the fact that conjugation rarely occurs if only a few individuals are present in a dish, it is difficult to determine just how long a time must elapse after conjugation before the ex-conjugants are able to conjugate again. Jennings (1910) found a case in which the descendants of a single ex-conjugant conjugated among themselves after only four divisions, with an interval of five days. In race 385–1, conjugation was observed among the descendants of an ex-conjugant ten days after reorganization was complete. In two other instances, several pairs of conjugants were placed together in the same dish. Reconjugation occurred among their descendants in seventeen days and in twenty days, respectively, fresh medium having been added in each case, three days previously.

Relation of conjugation to length of dormant period.—The occurrence of a starvation or dormant period of some length was found by Zweibaum (1912), Hopkins (1921), and others to increase the susceptibility toward conjugation. Zweibaum was unable to induce conjugation, even with favorable salts, in races that had not been subjected to this period of dormancy.

A comparison of the length of dormant period in race 385–1 with the percentage of instances of conjugation (table 13) showed that the number of cultures with conjugants was independent of the length of the dormant period. Cultures started from dishes that had received fresh food not more than ten days before had practically the same percentage of conjugations as did cultures started from dishes that had been dormant more than twenty days. The intensity of conjugation appeared to decrease with the length of the dormant period (table 13).

TABLE 13

EFFECT OF LENGTH OF DORMANT PERIOD ON THE RELATIVE NUMBER OF INSTANCES OF CONJUGATION AND ON ITS INTENSITY

[1 = conjugation 5% or less. 2 = conjugation 6–20%. 3 = conjugation 21–50%. 4 = conjugation more than 50%.]

Period without fresh food	Number of transfers	Number of instances of conjugation	Per cent of instances of conjugation	Average maximum intensity of conjugation
10 days or less	92	62	67.4%	2.08
11-20 days inclusive	29	21	72.4%	1.65
More than 20 days	28	19	67.9%	1.33

Experiments with vitamin X are omitted.

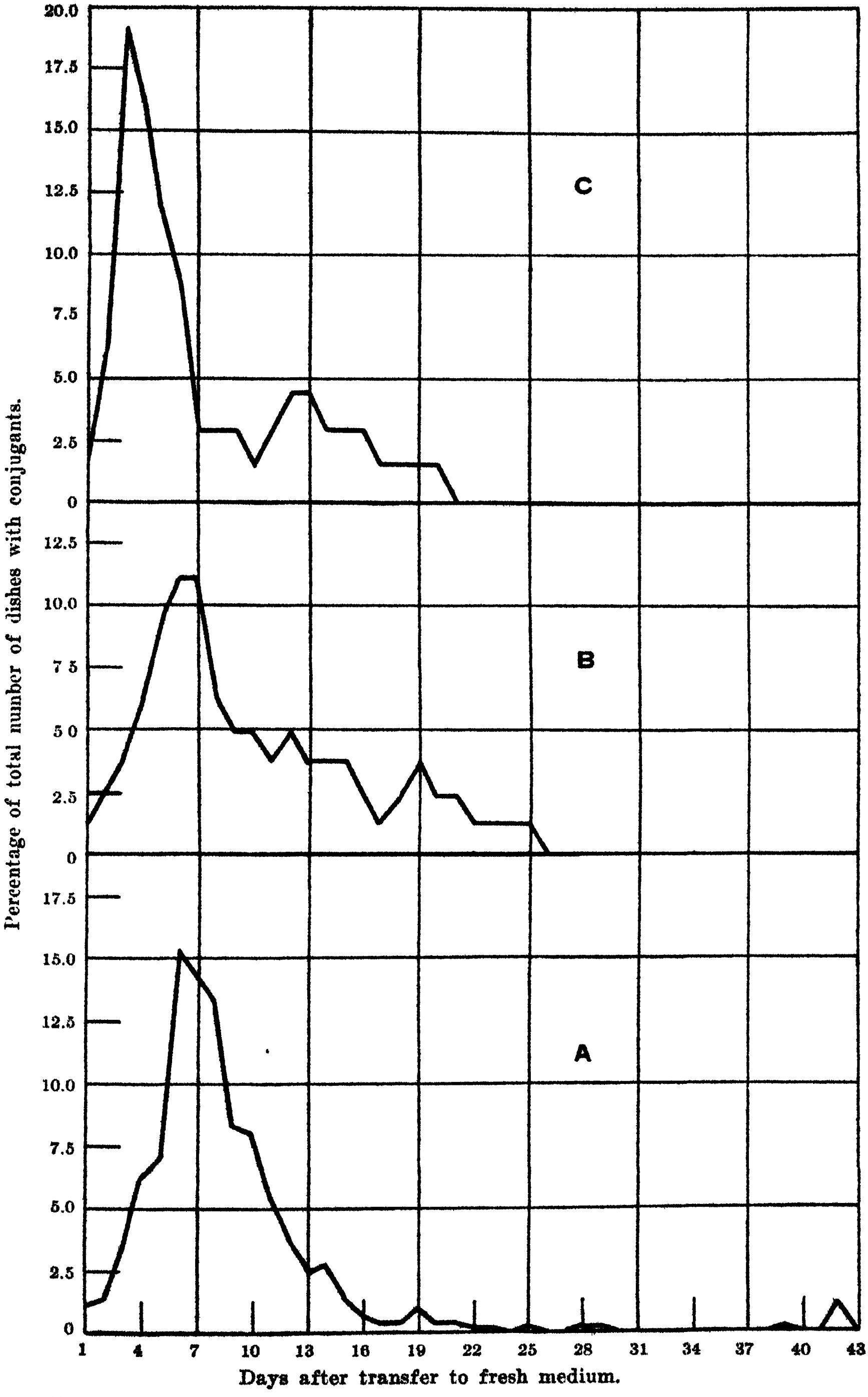

Fig. 3. Race 385–1, *P. caudatum.* Relation between length of dormant period and the number of days after transfer to fresh medium that conjugation occurred.

A—Cultures dormant 10 days or less.

B—Cultures dormant 11–20 days. C—Cultures dormant more than 20 days.

A graph of the relation between length of dormant period and the time after transfer to fresh food that conjugation occurred, showed that conjugation tended to appear sooner when the dormant period was longer (fig. 3). Cultures from dishes which had received fresh food not more than ten days previously gave the most conjugants on the sixth to the eighth day after transfer; those from dishes fed eleven to twenty days before had the most conjugants on the fifth to the seventh day; while those from dishes fed more than twenty days previously had the most conjugants on the third to the fifth day after transfer. In this race, therefore, an extended dormant period tends to bring about conjugation sooner if the organisms are transferred to fresh food than does a briefer period without the addition of fresh medium. On the other hand, the percentage of conjugating to non-conjugating cultures is not altered by the length of dormancy, although the number of conjugants in any one culture decreases with the extent of the dormant period. This may be due to the smaller number of animals present in the older cultures.

Relation of conjugation to the number of Paramecium.—Conjugants rarely appeared in a dish containing only a few *Paramecium*. Practically no instances of conjugation were found as late as the twenty-first day after transfer to fresh food; and very few occurred before the third day. The occurrence of a certain minimum number of animals in a dish is evidently necessary in order that they come in contact often enough to produce permanent unions. In this race, conjugation did not seem to occur at the beginning of a decline in rich food conditions (Jennings, 1910). On the contrary, the first conjugants usually appeared several days before the number of animals in the culture reached a maximum; and, in many instances, the epidemic of conjugation had been completed before the numbers of *Paramecium* in the culture had stopped increasing.

Considering only the first fourteen days of the culture, a comparison was made between the time after transfer to fresh food that conjugation appeared and the number of animals in the same cultures during a like period. For determining the number of animals, daily records based on an arbitrary grading scale were used. From these data, there is no evidence that conjugation tended to occur when food conditions were beginning to decline (fig. 4). In nearly three hundred cultures containing conjugants, the maximum number of animals tended to appear decidedly after the peak of the wave of conjugation had been reached. There was no noticeable decline in the number of animals up to the eleventh day; on the latter date, the percentage of conjugation was very low. In this race, conjugation appeared on the ascending portion of the curve for number of animals rather than on the crest or on the descending portion.

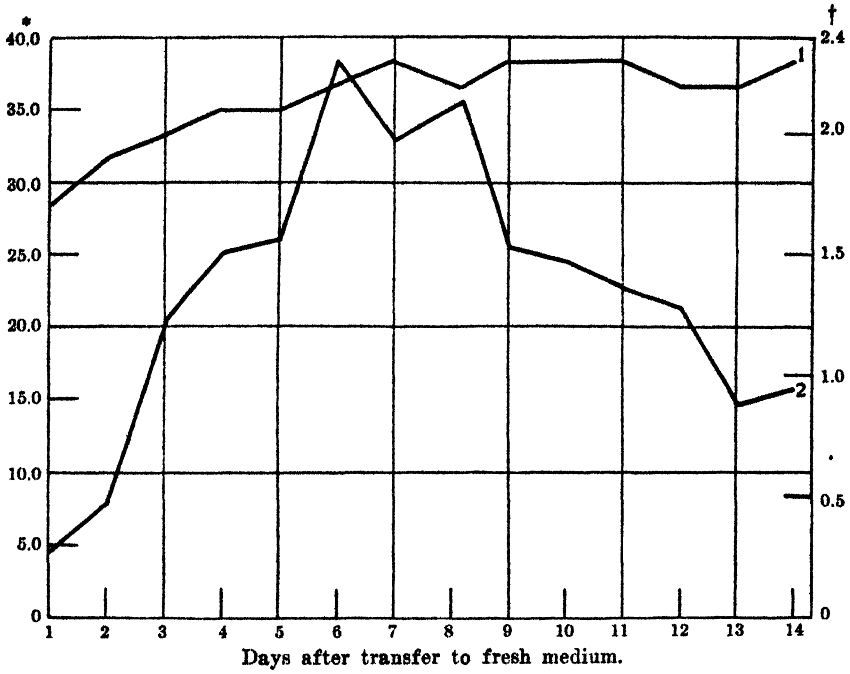

Fig. 4. Race 385–1, *P. caudatum*. Relation between the number of *Paramecium* in the cultures and the occurrence of conjugation for successive days after transfer to fresh medium.

1. Average number of animals in all the conjugating cultures, based on an arbitrary grading scale.
2. Distribution of conjugation for successive days after transfer to fresh medium.

* Percentage of dishes with conjugants to total number of dishes.
† Number of animals.

Relation of conjugation to size of culture dish.—Hopkins (1921) and others found that conjugation was more apt to occur in small-sized culture dishes than in large ones. In race 385–1, however, conjugation appeared to proceed with considerable independence of the size of the containing vessel. It was as apt to occur in stender dishes 5 cm. in diameter as in those two to three times as wide. On the other hand, conjugation occurred much less frequently in Petri dishes, in which the food seemed to be exhausted much more rapidly.

Relation of conjugation to character of medium.—In the Petri dishes mentioned above, the depth of the fluid is shallow, and the surface exposed to the air relatively large; there is little opportunity for the medium to become excessively acid. On the other hand, the stender-dish cultures always had a very high acidity. Infusions which were made alkaline by the addition of NaOH or $MgCO_3$, or which were kept at a constant pH by the addition of a piece of marble (Bodine, 1921), showed no appreciable alteration in their conjugation reactions.

TABLE 14

AVERAGE pH VALUES OF SIX DIFFERENT SETS OF CULTURES FOR EIGHT SUCCESSIVE DAYS AFTER TRANSFER TO FRESH MEDIUM. GLUCOSE ADDED TO MAKE CULTURES ACID. MARBLE AND $MgCO_3$ ADDED TO MAKE CULTURES ALKALINE

Days after transfer to fresh medium	Acid	Control	Alkaline
1	6.2	6.2	7.3
2 (glucose added)	6.4	6.4	7.5
3	5.7	6.5	7.7
4	5.2	6.3	7.9
5	4.8 (many dead)	6.2	7.8
6	4.8 (nearly all dead)	6.3	7.9
7	4.7 (all dead)	6.4	7.8
8	4.9	6.3	7.8

TABLE 15

DIFFERENT pH VALUES AT WHICH CONJUGATION OCCURRED

Race 385–1, *P. caudatum*

Line	Days after transfer that conjugation occurred	pH values of conjugating cultures	
A	2	6.4	7.4
A	3	6.7	
A	4	6.6	
A	5		8.1
B	4	6.0	7.4
C	6		7.5
C	7	6.9	
D	5	6.4	
D	6	6.4	
D	7	6.8	
D	8	6.4	
E	11		7.7, 7.8
F	11		7.2

In order to test this matter more thoroughly, each of several single cultures was divided up into sets of three serial cultures as follows: one culture was kept acid by the addition of glucose; one was kept alkaline by the addition of $MgCO_3$ and marble; and a third was permitted to run its normal course without attempting to alter the pH. The pH of each of the cultures for eight days following transfer to fresh medium was determined (table 14) by means of a Gillespie colorimeter (Clark, 1922, pp. 128–131). In addition, the pH readings were taken of other cultures in which conjugation occurred. There was no relation between the hydrogen ion concentration and the occurrence of conjugation, conjugants appearing with equal facility in cultures with pH values ranging from 6.0 to 8.1 (table 15). The average change in pH readings for eight successive days after transfer to fresh medium for six acid, six alkali, and six control cultures is shown in figure 5. A pH of less than 5.0 was fatal to *Paramecium* within one or two days.

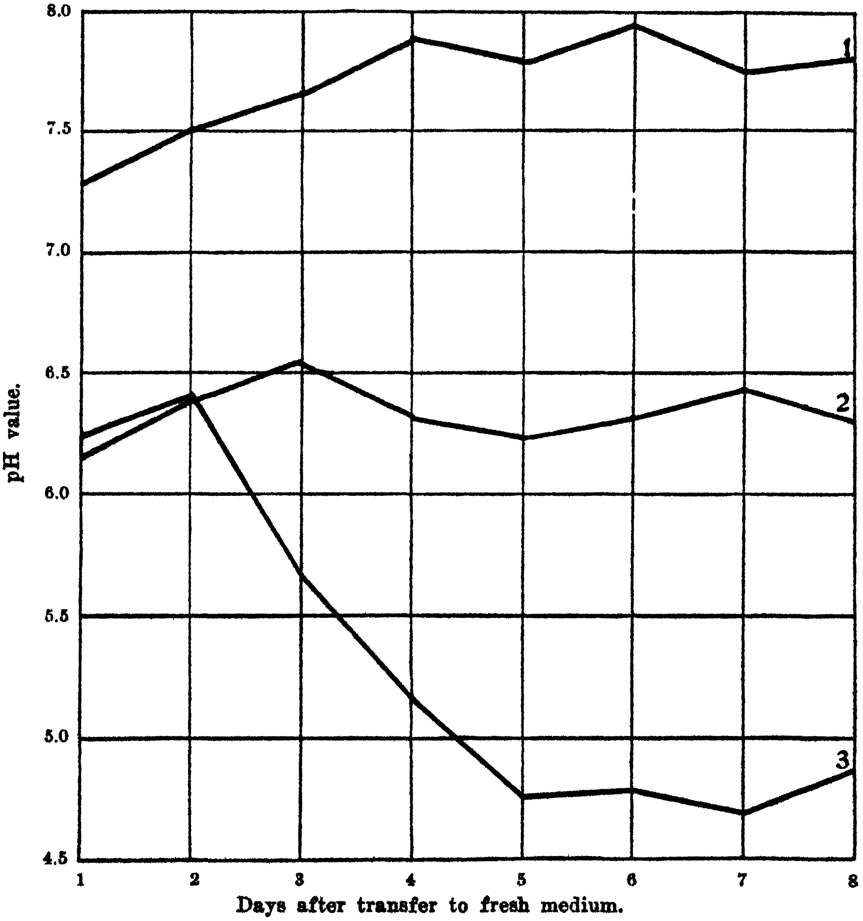

Fig. 5. Race 385–1, *P. caudatum*. Average pH values of six different series of cultures for eight successive days after transfer to fresh medium.

1. Average pH values for cultures to which marble and magnesium carbonate were added at the time of transfer to fresh medium.

2. Average pH values for control cultures.

3. Average pH values for cultures to which glucose was added the second day after transfer to fresh medium.

The character of the medium, however, does influence the occurrence of conjugation in this race. Through the courtesy of Dr. H. M. Evans of the Department of Anatomy, opportunity was offered to test this and other races of *Paramecium* with vitamin X (Evans and Bishop, 1923). Absence of this substance from the diet of female rats, it has been found, interferes with the process of reproduction. An aqueous extract was prepared from wheat embryo, and cultures of different races of *Paramecium* were fed this extract instead of hay infusion.

The use of vitamin X did not produce conjugation in any of the six races which rarely conjugated. On the other hand, although race 385–1 would conjugate when transferred directly from hay infusion to vitamin X (table 5), it lost its power to conjugate in this medium, or in solutions of $AlCl_3$, $FeCl_3$, or in distilled water, after being fed on vitamin X for six days. The results continued negative through three successive transfers although conjugation was occurring frequently at this time in sister cultures kept on hay infusion (table 16). After being on vitamin X for eighteen days, the organisms were transferred back to fresh hay infusion, whereupon they conjugated within three days. In subsequent transfers, this line did not differ from the other cultures in its conjugation reactions. It was hardly to be expected that vitamin X would increase the sexual activities of *Paramecium,* since its action is nutritional, that is, it prevents the absorption of the embryos *in utero,* and does not incite to sexual activity.

Enriques (1910) stated that culture media in which conjugating *Colpoda* were living would induce conjugation in a non-conjugating culture; and that fluid from a non-conjugating culture would stop an epidemic in a conjugating one. In order to see if any substance were present in a dish of conjugating *Paramecium* which would induce union in a race that was not conjugating, animals from non-conjugating cultures of other races were centrifuged and transferred to media from cultures of race 385–1, both before and during the occurrence of conjugation in the latter. Centrifuging does not, in itself, prevent the occurrence of conjugation, although it seems to delay its appearance. Transfer of other races to media from cultures of race 385–1 did not enhance the conjugating power of the former. On the other hand, members of race 385–1, which were contrifuged and transferred, both before and during conjugation, to media of non-conjugating cultures of other races did not lose their power of conjugation (table 17).

TABLE 16

Behavior of Different Races on Vitamin X

Race	Days after transfer to fresh medium that conjugation occurred			
	Fresh medium, Oct. 6, 1923	Fresh medium, Oct. 11, 1923	Fresh medium, Oct. 16, 1923	Fresh medium, Oct. 20, 1923
48–1:				
Hay	0	0	0	0
$AlCl_3$	0	0	6	0
$FeCl_3$	0	6	6	0
Distilled water	0	7–8	6–10	0
Vitamin X	......	0	0	0
364–1:				
Hay	0	0	0	0
Vitamin X	......	0	0	......
383–1:				
Hay	0	0	0	0
$AlCl_3$	0	0	0	0
$FeCl_3$	0		0	0
Distilled water	0	0	0	0
Vitamin X	0	0	0	0
384–1:				
Hay	5	0	0	0
$AlCl_3$	6	6	0	......
$FeCl_3$	6	6–8	0	0
Distilled water	0	6	6–7	0
Vitamin X		0	0	0
386–1:				
Hay	0	0	0	0
Vitamin X	0	0	0	0
387–1:				
Hay	0	0	0	0
$AlCl_3$	0	0	......	0
$FeCl_3$	0	0	0	0
Distilled water	0	0	......	0
Vitamin X	0	0	0	0
385–1:				
Hay	5–7	1, 5–8	1–2, 5–8	5–8
$AlCl_3$	6–7	6–7	6, 8	5
$FeCl_3$	6–9	6–8	6–7	5–6
Distilled water	6–7	6–8	6–8	5–7
Vitamin X	6–8 (from hay)	6–10 (from hay)	0 (4 cultures)	0 (7 cultures)

TABLE 17

Effect on Conjugation of Transferring Animals from Race 385–1 to Media of Other Strains; and of Transferring Animals of Other Strains to Media of Race 385–1

Animals	Media	When transferred	Subsequent behavior
158b″	385-1a	Before conjugation	No conjugation
385–1a	158b″	Before conjugation	Conjugation after 5 days
48–2	385–1b	During conjugation in 385–1b	No conjugation
48–2	48–2	During conjugation in 385–1b	No conjugation
385–1b	48–2	During conjugation in 385–1b	No conjugation
385–1b	385–1b	During conjugation in 385–1b	Conjugation after 2 days
383–1	385–1b	Before conjugation	Conjugation after 7 days
385–1b	383–1	Before conjugation	Conjugation after 2 days
386–1	385–1c	Before conjugation	No conjugation
385–1c	386–1	Before conjugation	Conjugation after 6 days
387–1	385–1d	Before conjugation	No conjugation
385–1d	387–1	Before conjugation	No conjugation
387–1	385–1e	During conjugation in 385–1e	No conjugation
387–1	387–1	During conjugation in 385–1e	No conjugation
385–1e	387–1	During conjugation in 385–1e	Conjugation 1st to 5th day
385–1e	385–1e	During conjugation in 385–1e	Conjugation 1st to 3d day

Relation of conjugation to bacterial flora.—The results of Chatton and Chatton (1923*a, b*) indicate a possible relationship between the kind of bacteria present in a culture and the occurrence of conjugation. The zygogenic media of these authors contained certain salts with definite kinds of bacteria. A medium which would induce conjugation in one instance was ineffectual when a different species of bacterium was added.

Since the occurrence of conjugation did not appear to be related to other environmental factors, it was possible that differences in bacterial flora might account for differences in the behavior of the various races; or else that the tendency for conjugation to occur within a limited number of days after transfer to fresh medium was connected with some cycle in the bacterial flora.

Beginning one day after transfer to fresh food, samples of media from different infusions were streaked on agar plates for eleven successive days. From these plates, single colonies were isolated and used for determining the staining reactions, motility, and behavior of the bacteria on other types of media. The bacterial flora appeared to be the same in the cultures of both 385–1 and other races, and no variation in the flora was observed in connection with the appearance of conjugation (table 18). In different races of 385–1, conjugation occurred at various intervals after transfer to fresh media, but there was no indication of any difference in the kind of bacteria present. Tests made from both old and young cultures gave similar results.

TABLE 18

CONJUGATING BEHAVIOR OF DIFFERENT LINES LIVING ON THE SAME KINDS OF BACTERIA

[Fresh medium March 4, 1924. Bacterial cultures made daily March 5–15 inclusive gave *Bacillus fluorescens mutabilis* in large numbers and *Bacillus ubiquitus* in small numbers, in all cultures. X = conjugation.]

Race	Days after transfer to fresh medium																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
385–1c	....	X	X	X	X	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
385–1a	X	....	....	....	....	X	X	....	....	X	....	X	X	....	X	....	....	....	X	X	X
385–1d	....	....	....	....	....	X	X	X	X	X	X	X	X	....	....	....	....	....	....	....	....
385–1b	No conjugation in 26 days.																				
g–2 (ex-conj. of 385–1)	....	X	X	X	X	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
387–1	....	....	....	....	....	....	....	....	....	....	....	....	....	X	X	....	....	....	....	....	....
364–1	No conjugation in 26 days.																				

It was found that the hay infusions were practically pure cultures of *Bacillus fluorescens,* belonging probably to the variety *B. fluorescens mutabilis,* described by Wright (1895). Organisms of the *fluorescens* group have the power of inhibiting the multiplication of other bacteria; this perhaps accounts for the occurrence of the group in practically pure culture. In addition, all the cultures examined had a small number of organisms belonging to another species of bacteria, which corresponded closely to *Bacillus ubiquitus* Jordan (1890). These were the only species of bacteria present in any significant numbers. Cultures of one-day-old hay infusion showed occasionally a few other bacteria, such as *B. subtilis,* but the subsequent growth of these species appeared to be inhibited by the dominance of *B. fluorescens.* It is interesting that Chatton and Chatton (1923*a*, *b*) found that *B. fluorescens* induced conjugation in *Colpidium colpoda,* but prevented it in *Glaucoma scintillans.*

GENERAL DISCUSSION

The organisms with which Zweibaum (1912) worked all belonged to the same clone (*"Les cultures en expérience dérivent toutes d'un seul individu,"* p. 277). Without adequate testing, an attempt to apply results obtained with only one clone of *Paramecium* to all members of a species is dangerous in the face of the findings of Jennings (1910) and of Jennings and Hargitt (1910) that a population of *Paramecium* consists of a large number of clones with different structural and physiological properties. Among these are differences in the tendency to conjugate. Hopkins (1921) showed that Zweibaum's technique would not induce conjugation in all races. The present work is a further confirmation of the fact that conditions for conjugation are different for different clones of *Paramecium.* This is probably the explanation of the fact that methods for inducing conjugation which have been recommended by one worker frequently fail when tested by others.

The principal result of this attempt to induce conjugation in *Paramecium* by a repetition of Zweibaum's method has been the discovery of a race of *P. caudatum* with a marked tendency to conjugate under certain conditions. These conditions are not the same as those which Zweibaum found effective for his race, and could not be applied, in turn, with positive results to the other races tested in the course of the present experiments. In some respects, the conditions for conjugation in this race differ from those which have been described for the majority of other "conjugating races." The race of *Chilodon uncinatus* described by Enriques (1916) appears to have exhibited a similar behavior, reconjugating as early as two days after the addition of fresh medium. In this race, conjugation was largely independent of either external or internal conditions.

In the first place, the power to conjugate is not accelerated by a long period of dormancy (table 13). There is no evidence, in this race, that the power to conjugate is connected with an abrupt rise in the rate of fission. A good proportion of the instances of conjugation occurred in a series of cultures which were transferred to fresh food every five or six days. In these cultures, there was no opportunity for the division rate to decrease to any great extent.

Furthermore, the occurrence of conjugation in this race almost continuously for several months, and the power to obtain it at practically any time by a transfer to fresh media, gave no evidence of any inherent tendency of the race to conjugate in fixed cycles of a definite length. Nevertheless, it was impossible to know whether the animals which conjugated at one period were descended from those which had conjugated five or six days previously, or whether they were derived from the non-conjugants of the preceding period. In the latter case, conjugation might be occurring in cycles, but this fact would not be apparent due to the occurrence in the culture of several different cycles of conjugation, coming to expression at different times.

In cultures derived from ex-conjugants or from conjugating pairs, a greater interval of time seems to be required before conjugants again appear than is the case in the main cultures. This is due to the fact that a longer period is necessary before the animals become numerous enough to insure conjugation. Nevertheless, it was possible to obtain reconjugation in cultures derived from a single ex-conjugant ten days after the reorganization process was completed. In another case, five pairs of conjugants were placed in the same dish; the descendants of these were conjugating again in seventeen days; a similar method employed with twenty pairs of conjugants gave reconjugation on the twentieth day. These results do not indicate the presence in this race of a cycle of conjugation of from four to twelve weeks, as was found by Hopkins (1921) for his races of *P. caudatum.*

Another peculiarity of this race was that conjugation was induced not by starvation, but by transfer to a fresh and rich food supply. In this respect, the race resembled some of those used by Hopkins, in that conjugants appeared with equal facility in the main cultures, which had received fresh food without dilution, and in the subcultures, which had been transferred to distilled water or to salt solutions. Starvation, in the form either of dormancy preceding transfer to fresh food or of a sudden reduction in the food supply after a brief period on rich medium, did not increase the power of conjugation.

The behavior of this race of *P. caudatum* also resembles in many respects the *k* race of *P. aurelia* described by Jennings (1910). In the latter race, a brief dormant period of from two to three weeks was usually required between two epidemics of conjugation, yet, when conjugants were obtained, they appeared usually a few days after the addition of fresh food. In some instances, this occurred as early as two days after the fresh infusion was added. From measurements of the animals, Jennings concluded that conjugation occurred at the time food conditions were beginning to decline, although multiplication might still be continuing. Observations on race 385–1 showed that the maximum number of animals in a culture was reached after the crest of the conjugation epidemic. Furthermore, at the time the number of organisms was beginning to decrease, there were very few conjugants in the culture. From figure 4 it may be concluded that conjugation tended to occur at a time when the division rate was increasing less rapidly, but there was no indication that conjugation was connected with a beginning of a starvation period.

As the culture progressed, there was a tendency for conjugation to occur with less regularity at a definite number of days after transfer to fresh media (fig. 2). This phenomenon has a possible genetic explanation. The appearance of hereditarily diverse lines in a clone of *Paramecium* as a result of conjugation was noted by Calkins and Gregory (1913), Jennings (1913), Stocking (1915), Hopkins (1921), and Jollos (1921). A similar process was shown to occur at the time of endomixis by Erdmann (1920). Reference to table 12 shows that the ex-conjugants, in race 385–1, differed from one another in their ability to conjugate under similar conditions. It is possible that there occurred a similar splitting up of the clone regarding its power of conjugating at definite intervals after transfer to fresh media. The conditions under which the organisms were kept were believed to be constant throughout the experiment. There could be no question of acclimation to laboratory conditions, since this race had already been living in the laboratory for at least sixteen months before these experiments were begun. Since the clone was known to have split up in respect to other physiological properties, i.e., power of conjugation, it is not improbable that there is a similar explanation for this flattening of the curve of conjugation. It will be of interest to observe the future behavior of this race; to see if the curve continues to flatten out, and if the race continues to retain its powers of conjugation.

Are the conditions for conjugation in *Paramecium* external or internal? They are not purely external, since conditions which succeed with one race frequently will not work with others. Medium from a conjugating culture of race 385–1 will not induce conjugation in other lines. Nor are the causes purely internal; "conjugating races" do not conjugate unless the proper environmental "conditions for conjugation" are supplied. Race 385–1 conjugated on hay infusion but not on vitamin X. Conjugation depends upon a proper combination of various internal and external factors. Starvation will not induce conjugation in a race that conjugates only when it is well fed. On the other hand, such a race will conjugate on certain types of food but not on others. Neither internal nor external conditions are the more important; both are indispensable.

SUMMARY

1. Experiments were performed in order to see if Zweibaum's method of inducing conjugation in one race of *Paramecium caudatum* would be successful with other races, both of *P. aurelia* and of *P. caudatum.* The results were essentially negative for eight out of the nine clones used.

For clone 385–1:

2. In the ninth clone (385–1, *Paramecium caudatum*), conjugation appeared regularly upon transfer of the organisms from a rich infusion, in which they had been living for from five to six days, to solutions of $AlCl_3$ N/10000 and $FeCl_3$ N/15000–N/18000. This was the method used by Zweibaum.

3. Conjugation appeared as frequently and with equal intensity in cultures diluted with distilled water as in those transferred to solutions of $AlCl_3$ or of $FeCl_3$.

4. Conjugation appeared as frequently and with equal intensity in cultures fed with fresh food and not diluted, as in those subjected to dilution with distilled water or transferred to solutions of the abovementioned salts.

5. The majority of instances of conjugation occurred from the fourth to the ninth day after transfer to fresh food, with the greatest number on the sixth day.

6. Subjecting this race for nearly seven months to the conditions of the experiment did not destroy its powers of conjugation. For three successive months, cultures were transferred to fresh food on an average of once every five to six days, and they gave conjugants on practically every transfer (table 8). In the entire series of experiments, excluding those on vitamin X and the ex-conjugant cultures, conjugation occurred in 68.5 per cent of the dishes inoculated.

7. There was no evidence for a cycle of conjugation; cultures derived from ex-conjugants showed conjugation ten days after the latter had completed the process of reorganization. Cultures derived solely from conjugating pairs were conjugating again in from seventeen to twenty days.

8. Cultures derived from different ex-conjugants differed in their conjugating behavior. Conjugation appeared to reduce or to destroy the conjugating power in some instances but not to affect it in others.

9. As the experiment progressed, conjugation appeared at much more irregular intervals after transfer to fresh food than was true in the early part of the work (fig. 2). A possible genetic explanation of this is suggested, based on the breaking up of the clone in conjugation or endomixis.

10. An extended dormant period did not increase the percentage of conjugation or its intensity, but it did cause conjugation to appear sooner than it occurred in cultures started after a briefer dormant period.

11. A dormant period of six weeks did not result in a loss of the power to conjugate upon transfer to fresh food.

12. Conjugation tended to occur in a culture before the maximum number of animals was reached and disappeared before the number of animals began to decline.

13. Within wide limits, conjugation was not related to the size of the culture dish.

14. Conjugation was apparently unrelated to the hydrogen ion concentration of the medium or to the kind of bacteria present.

15. This race lost its power to conjugate when transferred to vitamin X, but regained it when again placed on hay infusion.

16. Other races transferred to medium from a conjugating culture of 385–1 did not have their powers of conjugation increased; and race 385–1 conjugated when transferred to media of non-conjugating cultures of other races.

17. The conditions for conjugation in this and in other races of *Paramecium* depend upon a certain combination of internal and external factors. Conditions true for one race do not necessarily hold for others.

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KARYAMOEBINA SUBSTITUTED FOR KARYAMOEBA, WITH A NOTE ON ITS OCCURRENCE IN MAN

BY

CHARLES A. KOFOID AND OLIVE SWEZY

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KARYAMOEBINA SUBSTITUTED FOR KARYAMOEBA, WITH A NOTE ON ITS OCCURRENCE IN MAN

BY

CHARLES A. KOFOID AND OLIVE SWEZY

In volume 26, pp. 221–242, of the University of California Publications in Zoology we described an amoeba from the digestive tract of man under the generic name of *Karyamoeba*. Dr. C. W. Stiles has called our attention to the prior use of this name by Giglio-Tos (1900) for an amoeba found by him in the renal tubules of the rat and named *Karyamoeba renis*. It therefore becomes necessary under the rules of zoological nomenclature to provide a new generic name in place of *Karyamoeba*. We therefore propose *Karyamoebina,* a name which preserves much of the name first proposed and also retains an indication of the relationship of the genus. The type species is *Karyamoebina falcata* (Kofoid and Swezy, 1924).

Since the publication of the original description, at which time only three cases of infection had been detected, we have found three other cases of infection in man. The first of these was Mr. O, age 53, six years resident in Manila, P. I, from 1899 to 1905, and since then in California. In this case there were concomitant infections by *Endamoeba dysenteriae, E. coli,* and *Councilmania lafleuri*. *Karyamoeba* persisted in this case after treatment for amoebiasis with emetine-bismuth-iodide.

The second case was a resident of Oregon and California, Mrs. J, age over 40. In this case also there were concomitant infections by *Endamoeba dysenteriae* and *Endolimax nana*.

The third case was Mr. E, age 52, resident in Illinois, Idaho, and California. No concomitant infections have been found thus far in this case.

The total number of stool examinations made by us in the period since our first finding of *Karyamoeba* to date is 13,894. The total number of persons examined in this period is not less than 3000. The incidence of infection by this amoeba is thus very light. It appears to resist treatment for amoebiasis and to persist in some instances, but to be very erratic in abundance and therefore to be erratic in records of occurrence under ordinary conditions of stool examination. Its persistenee in the host, or recurrence in stools, and our findings of six cases of infection in the past sixteen months, is indicative of its being a normal inhabitant of the human bowel, but relatively rare.

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CRYPTOTERMES HERMSI SP. NOV.
A TERMITE FROM FANNING ISLAND

BY

HAROLD KIRBY, Jr.

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CRYPTOTERMES HERMSI SP. NOV. A TERMITE FROM FANNING ISLAND

BY

HAROLD KIRBY, JR.

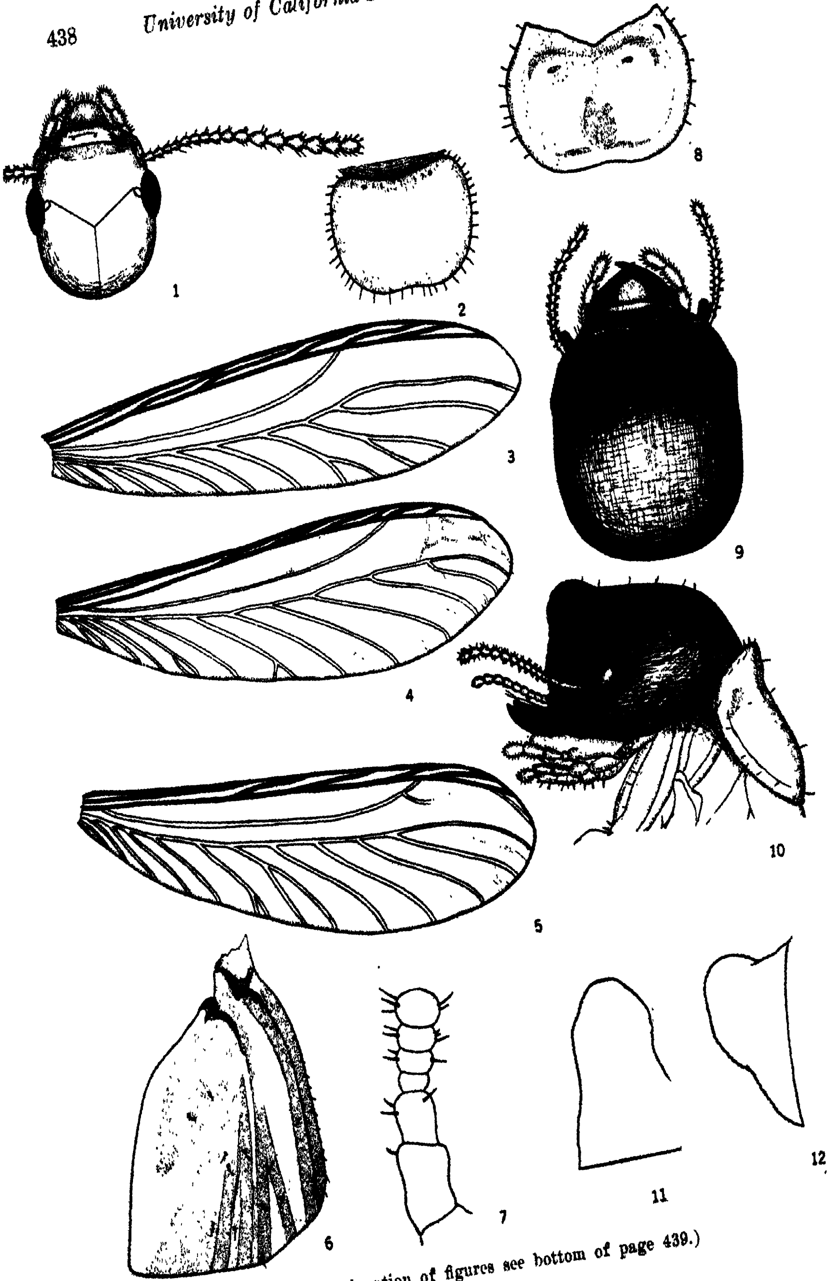
A termite, hitherto undescribed, collected at Fanning Island, Central Pacific, in the summer of 1924, has been named in honor of Professor W. B. Herms, with whom the writer was then associated, and through whose kindness it was possible to secure the material. Thanks are due to Mr. S. F. Light, who gave invaluable assistance during the study of this termite and furnished indispensable comparative specimens.

The insect is common at Fanning Island in decayed logs and stumps of coconut and *Tournefortia.* Winged adults were abundant at light in May and June and were collected from logs until August.

Winged adult (figs. 1–7).—The general color is yellow-brown, the head darker than the remainder of the body. The thoracic and abdominal tergites are yellow-brown, the ventral surface yellowish white. The pronotum has a narrow, red-brown anterior margin, and there is a median red-brown line on the meso- and metanotum. The wings are hyaline, with the chitinized marginal veins yellow-brown and the median and cubitus veins indistinct. The eyes are reddish black.

The head is quadrate oval in shape, longer than broad. The compound eyes are relatively large and prominent; the ocelli small, subtriangular, in contact with the eyes at about the middle. The antennae are long with 14–17 joints; labrum yellow and rounded as in the other species. The pronotum is large and shaped much as in *Cryptotermes kotoensis,* but the anterior concavity is deeper and the structure is not so wide, so that it is less oblong in appearance. The posterior margin is slightly concave, the sides rounded.

The wings are long, longer than those of *Cryptotermes kotoensis* Oshima, which this species closely resembles in size and morphology. The wing venation is much the same in both, but the median vein



(For explanation of figures see bottom of page 439.)

usually joins the radial sector more distally than in Oshima's species, in the outer third of the wing. There is, however, much variation in the wing venation.

MEASUREMENTS OF *Cryptotermes hermsi* SP. NOV., ADULT

(In millimeters, of alcoholic specimens.)

Length of body to tip of wings	8.86–9.62
Length of body without wings	4.53–5.45
Length of forewing	7.23–7.90
Length of head	1.11–1.22
Width of head (across eyes)	.89–1.00
Length of antennae	1.28–1.78
Length of pronotum (in midline)	.61
Width of pronotum	.95–1.11
Maximum diameter of eye	.24

Soldier (figs. 8–12).—The front of head, mandibles, and anterior dorsal part is black, shading on sides and top through purplish brown into yellow-brown. The antennae are yellowish white, the legs and body yellow-brown.

The head is at an angle to the axis of the body, the forehead high and protruding, making less than a right angle with the mandibles. The front is not deeply excavated, but is somewhat convex except for the dorsal and lateral projecting margins. The anterior and front-upper surfaces are much roughened, though by no means so much so as in *Cryptotermes piceatus* Snyder. The anterior margin as seen dorsally is convex, somewhat uneven and shallowly notched in the midline. As seen from the front there is a shallow concavity in the center. The antennal spine is stout, higher than wide, prominent, slightly upturned, and has a finely crenate margin, due to a fine corrugation of the surface. The dorsal margin is elevated anteriorly, as

---

EXPLANATION OF FIGURES

Structure of adult and soldier of *Cryptotermes hermsi* sp. nov. All drawings made with camera lucida from alcoholic material.

Fig. 1. Head of adult. × 25.
Fig. 2. Pronotum of adult. × 25.
Fig. 3. Forewing. × 12.5.
Fig. 4. Hind wing. × 12.5.
Fig. 5. Hind wing. × 12.5.
Fig. 6. Wing base of right forewing. × 47.
Fig. 7. Basal segments of antenna of soldier. × 93.
Fig. 8. Pronotum of soldier. × 25.
Fig. 9. Dorsal view of head of soldier. × 25.
Fig. 10. Lateral view of head and prothorax of soldier. × 25.
Fig. 11. Dorsal view of antennal spine. × 93.
Fig. 12. Lateral view of antennal spine. × 93.

seen in profile in figure 10. The sides of the head are slightly curved, parallel, the anterior portion is as wide as the posterior (thus differing from *C. campbelli* Light). The head is almost as wide as long, about two-thirds as high as long.

The length of the protruding portion of the mandibles is more than half the height of the head; the mandibles are stout, curved at the tip, and toothed. The antennae possess 13 or 14 joints, rarely 15. The first segment is large, oblong; the second much smaller and of similar shape, or clavate; the third narrowest and glabrous; the fourth slightly wider and broader, and the more distal ones increasing in size.

The pronotum is bent up anteriorly, where it overlaps the back of the head; the sides are broadly rounded to the two anterior points, between which there is a straight-sided notch. In some the sides are less broadly curved. The posterior margin is shallowly concave.

The yellow eyespot is distinct, oval, oblique, about its own length posterior to the base of the antenna.

MEASUREMENTS OF *Cryptotermes hermsi* SP. NOV., SOLDIER

(In millimeters, of alcoholic specimens.)

Length of head, to upper margin	1.45
Length of head, to tip of mandibles	1.78
Length of exposed portion of right mandible	.67
Same of left mandible	.61
Length of antennal spine	.22
Height of head, anterior	1.00
Width of head	1.33–1.39
Width of pronotum, between anterior points	.89
Greatest width of pronotum	1.28–1.33
Length of pronotum in midline	.75
Greatest length of pronotum	1.00
Length of antenna	.95–1.17
Total length	5.28

Systematic position.—*Cryptotermes hermsi* is closely related to *C. kotoensis* Oshima, *C. campbelli* Light, and *C. domesticus* Haviland. Through the kindness of Mr. Light I was able to compare all these species with specimens as well as from descriptions. The described species differs from *C. campbelli* Light and *C. kotoensis* Oshima in that, in the soldier, the head is more elevated at the upper margin, and is broader anteriorly; the eyespot is larger and closer to the base of the antenna, while in the other two, especially in *C. kotoensis*, the eyespot is narrow, hardly more than a line, and obscure (in specimens); the antennal spine is broader at the base than in the other two species, in which it protrudes more finger-like; the notching of the pronotum is deeper and the whole is larger; and the head laterally and posteriorly is lighter in *C. hermsi* than in *C. campbelli* and *C. kotoensis.* The adult wing is longer than that of *C. kotoensis,* and the wing venation and pronotum differ as has been remarked. *C. domesticus* Haviland is of much smaller size and the compound eye is not so large in proportion to the size of the head.

It is very interesting to find that such a close relative of the oriental termites occurs at Fanning Island, which is near Hawaii and is visited by ships from those islands and Australia, from which localities no termites of the *kotoensis* group have been reported. *Cryptotermes hermsi* differs markedly from *C. primus* Hill of Australia and even more decidedly from *C. piceatus* Snyder of the Hawaiian Islands.

A study of the intestinal flagellates symbiotic with *C. hermsi* will appear shortly, preliminary to a more general study of the Protozoa living in the termites of the genus *Cryptotermes*, a study which should lead to some very interesting conclusions regarding the relationships of the hosts.

Type locality.—Fanning Islands.

A series of winged and deälated adults, soldiers, and nymphs collected from *Cocos* and *Tournefortia* stumps in May and August, 1924.

Type.—Winged adult male, in U. S. National Museum.

Cotypes in private collection, in the California Academy of Sciences, San Francisco, California, and in the Department of Zoology, University of California.

ZOOLOGICAL LABORATORY, UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA.

Transmitted February 7, 1925.

IMPROVED MICROMANIPULATION APPARATUS

BY

CHARLES VINCENT TAYLOR

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Within recent years it has become increasingly evident that many experimental studies on living matter require apparatus of the utmost refinement and precise control. For this purpose the method of micromanipulation has come to rank as an invaluable aid. The use of very fine glass-needles and pipettes which are manipulated in a moist chamber on the microscope by a three-way holder makes possible biological experimentation under the highest magnification of the microscope.

There is already considerable literature on the method itself as well as on the data resulting from its application (Barber, 1914; Chambers, 1922; Peterfi, 1922; Seifriz, 1921; Taylor, 1920; and others). These contributions have served to emphasize that refinement of technique is as indispensable to progress in biology as it is in the physical sciences and that the hand no less than the eye requires mechanical aids of finest precision. Barber, Chambers, and Peterfi have each designed micromanipulators that afford splendid control of microneedles and micropipettes. They have described also other apparatus and various methods for their construction and use.

In general principle the instrument described in the present article is similar to the micromanipulators designed by Barber and by Peterfi. By means of exact sliding parts and adequate compensating adjustments, this apparatus affords the three-way movement of very fine glass-needles, micropipettes, or microelectrodes, with perfect and continuous control under the oil-immersion objectives. Chambers' micromanipulator consists essentially of rigid bars that are "screwed apart against springs."

Precise control of the operative instruments (the cardinal requirement in micromanipulation) is obtainable more or less satisfactorily with the use of any of the manipulators mentioned above. Barber's apparatus, however, is subject to ready wear and maladjustments which may soon seriously impair accurate movements. This is due chiefly to lack of adequate contact surfaces of sliding parts. Peterfi has apparently largely overcome this deficiency in his model but has incorporated both fine and coarse adjustments and other accessories which would seem to approach considerable complexity. Chambers has sought to avoid undue wear and complexity in his manipulator by making use of springs instead of slides. The springs hold together rigid bars which are spread apart by means of thumbscrews. Disc-like guides are employed to maintain accurate travel of the moving bars. The guides require careful adjustment and occasional readjustment to correct a possible shift of the needle or pipette when the direction of movement is reversed.

The new pattern of micromanipulator herein described was designed with a view toward obviating several deficiencies, even some of minor importance, which have become apparent in other models. Chiefly because of its ample mass, which affords necessary stability to the extensive contact surfaces of its sliding parts, and to its simplified design and superior workmanship, which render easy its manipulation and give precision in its adjustments, this apparatus is believed to represent certain distinct improvements over other micromanipulators. It not only insures accuracy in each of its three movements but in recent tests has maintained that accuracy without readjustment through several months of more or less continuous use by beginning students. This durability is secured through the adequate mass of movable parts, all of which, because of simple pattern, are well protected from accidental knocks.

Another important advantage in this stable instrument is the provision for the manipulation of a new and much simplified micropipette apparatus (described below), which can be operated directly on the manipulator without producing any vibration of the pipette tip, that is in the least perceptible under the oil-immersion objective.

Stability and precision are further subserved by means of an amply spacious metal base (*c.i.b.*, pl. 34), which supports the right and left manipulators as well as the microscope. Two clamp screws (*m.*, pl. 34) hold the microscope rigidly in position midway between the manipulators (pl. 34). The latter, also, are firmly fixed in place by clamp screws (*c.s.*, pl. 34). Upon loosening these screws, however, each manipulator may be rotated through 90 degrees, thereby very conveniently providing for the substitution of new needle-tips or other operative instruments, or for their exchange without danger of breakage or involving the loss of time.

ACKNOWLEDGMENTS

Funds for the materials and construction of the apparatus described in the following pages were generously provided through a special research grant by President W. W. Campbell and the Research Board of the University of California, to whom acknowledgments are hereby gratefully made. I take this opportunity to thank, also, Mr. Vladimar Arntzen, Mechanician in the Department of Civil Engineering of the University of California, whose excellent skill and untiring labor are responsible for the practical development and construction of the apparatus.

THE MICROMANIPULATOR

This apparatus consists essentially of a right and a left manipulator (one the mirror image of the other) and a cast-iron base (*c.i.b.*, pl. 34). The latter supports the two manipulators and the microscope which is placed midway between them. The height of the manipulators (practically that of the microscope stage) and the distance between them are adjustable for any of the usual makes of microscopes. The base of each manipulator consists of a circular turntable (*tt.*, pl. 34), 14 cm. in diameter, which is assembled accurately with a central disk that is screwed solidly to the cast-iron base. The turntable is clamped in place by means of a clampscrew (*c.s.*, pl. 34). The latter passes through a slot (*s.*, pl. 34) near the periphery of the turntable, so that, upon loosening the clamp, the manipulator may be turned through 90 degrees. This very conveniently provides for ready access to the operative instruments without disturbing the microscope or moist chamber, thereby obviating risk of breakage or loss of time in changing or exchanging any of the delicate instruments.

The sliding parts (*sl.*, fig. A), which are made of bronze, include three pairs for each manipulator. The average length of each slide is 9 cm., and the width is 5 cm., giving for each pair of slides 45 sq. cm. of contact surface. This extensive contact surface together with its exact assemblage (obtained by hand finish) accounts for the perfect precision of all three movements. A steel gib (*g.*, fig. A) is introduced on one side between each pair of slides. This gib is regulated accurately by three screws (*g.s.*, fig. A) each of which works against a stiff spring. Continuous adjustment during any wear of sliding parts is thereby automatically maintained.

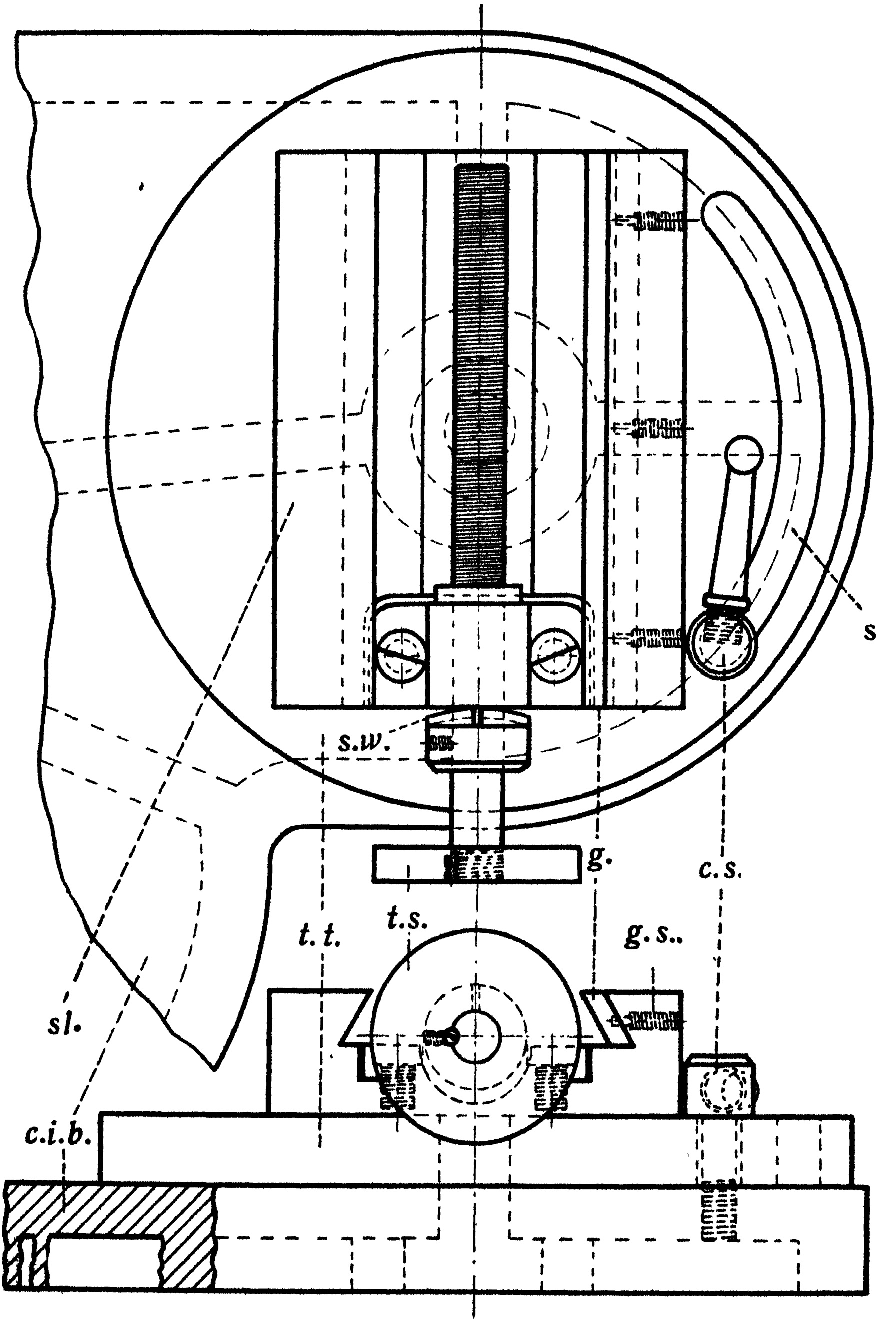

Fig. A. Details of micromanipulator: *c.i.b.*, cast-iron base; *c.s.*, clampscrews of turntable; *g.*, gib; *g.s.*, gibscrew; *s.*, slot in turntable; *sl.*, slide of manipulator; *s.w.*, steel washer of thumbscrew.

The top of the up-and-down slide of each manipulator projects 5 cm. at right angles toward the microscope to form a substantial arm (*a.*, pl. 34) which supports a movable holder (*h.*, pl. 34) for the operative instrument. The square base of the holder fits neatly into a slot which is provided with two clampscrews. The holder may be moved to any convenient position along the arm and there clamped firmly in place.

Three finely threaded steel screws (*t.s.*, pl. 34; fig. A) control the movements of the sliding parts. Each screw has a diameter of 9 mm. and is threaded 60 turns to the inch. The milled heads of the screws are 3.5 cm. in diameter. This convenient size of both the screw and its milled head facilitates accurate control of the operative parts.

Between the bearing and the capstan which hold the screw in place is introduced a convex steel washer (*s.w.*, fig. A) against which the capstan is firmly set. This affords a unique and very advantageous arrangement for maintaining automatically the exact assemblage of the screw and sliding part.

A SIMPLIFIED MICROPIPETTE

Several types of micropipettes for the injection or extraction of very minute amounts into or from protoplasm have been described by Barber (1914), Chambers (1921), Peterfi (1922), and Taylor (1920). The Barber and the Peterfi pipettes involve the principle of varying by means of heat a suitable volume of mercury contained in a glass tube on one end of which is drawn the pipette. Barber controls the expansion of the mercury by means of warm water. Peterfi expands the mercury by electrically heating a platinum coil which is contained in a bulbed end of the glass tube. The mercury is readily cooled as desired by introducing air into the bulb through a stopcock.

Peterfi's ingenious device, which I have not used, may obviate the difficulty encountered in the Barber micropipette; namely, the effecting by means of heat, of an adequate and sufficiently prompt control of the meniscus of mercury in an exceedingly fine pipette tip.

Chambers (1922) has recently made use of a carefully selected Luer glass syringe for controlling delicate movements of the fluid in the pipette tip. The syringe and pipette (the latter supported on his micromanipulator) are connected by a piece of small, flexible brass tubing, and the system is filled with reboiled distilled water.

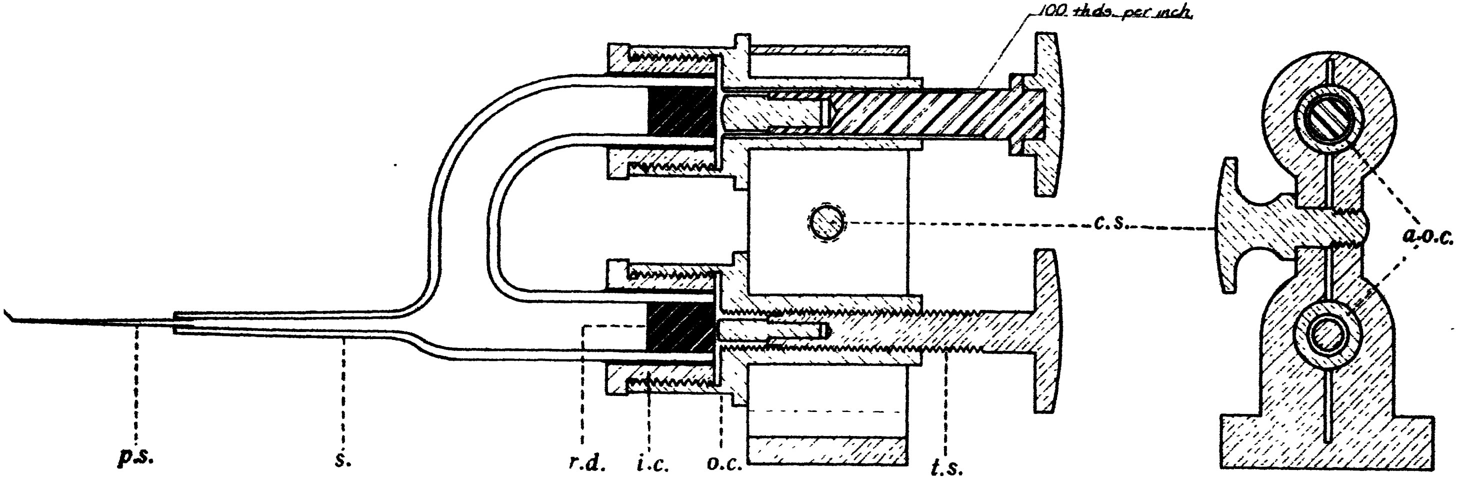

Fig. B. Details of micromanipulator: *a.o.c.*, arms of outer cap; *c.s.*, clampscrews; *i.c.*, inner cap; *o.c.*, outer cap; *p.s.*, pipette shaft; *s.*, pipette shank; *r.d.*, rubber diaphragm; *t.s.*, thumbscrew.

This device has the distinctly practical advantage of being easily and inexpensively made and it gives very satisfactory results. The water system requires recharging, however, after it has stood some hours.

The micropipette which I previously (1920) described depended upon the principle of displacing mercury in an enclosed system by means of a small steel needle. The needle projected through a close-fitting rubber plug into the column of mercury. This method finally proved to be somewhat impractical, however, because of the difficulty of preventing eventual leakage of mercury through gradual abrasion of rubber around the steel needle.

In that paper, I introduced the very convenient procedure of sealing the micropipette into a main shank by means of de Khotinsky cement. Others (see Chambers, 1921), also, have since found this to be an invaluable, time-saving method.

The simple micropipette now to be described has been tested for more than a year with excellent results. In principle, it involves varying the pressure on an enclosed volume of mercury or water by means of two screws which operate against thick rubber diaphragms. This device is not only simple and inexpensive in construction but it maintains for months perfect response to exceedingly delicate changes in pressure without readjustments other than the occasional replacement of the pipette shaft—a procedure which is quickly and easily accomplished.

The glass parts of the device (see fig. B) consist of a shank made in the form of the small letter "h," and the pipette shaft (*p.s.*) which is sealed with de Khotinsky cement into the tapering end of the shank. Into each of the basal ends of the shank is cemented a neatly fitting, thick rubber diaphragm. Onto each basal end is then sealed a bronze inner cap (*i.c.*). An outer cap (*o.c.*), which includes the thumbscrews (*t.s.*), is in turn screwed onto each of the inner caps. The pipette-holder supports the arms (*a.o.c.*) of the outer caps, which are rigidly fixed in place by means of a clampscrew (*c.s.*).

Before sealing the pipette shaft into the shank the latter is filled with either mercury or water. For most purposes I have found the use of mercury preferable, but it is very essential to have both the mercury and the glass parts scrupulously clean (Taylor, 1920). Another essential precaution is the avoidance of air bubbles anywhere within the filled system. To obtain the best results it is advisable to evacuate the air from the shank by means of a good vacuum pump. The mercury may then be introduced through a glass tube which is sealed at right angles into the tube leading to the pump. The free end of the T-tube is drawn to a rather fine slender tip about two inches long. About one inch of the tip is then bent at right angles and the end closed by melting in the flame. After the system has been well evacuated and the glass tube leading to the pump sealed off, the outer bend of the tip of the T-tube is carefully scratched with a file or diamond point. Thereupon the tip is broken under a goodly supply of *very clean* mercury by gently pressing the end against the bottom of the vessel containing the mercury, and the system thus completely filled. The pipette shank is now removed from the tube which led to the pump by *carefully* melting the cement in the joint. (Much caution is necessary in this procedure to avoid boiling the cement in the joint and so contaminating the mercury with the gas thus produced). By turning the thumbscrews against the diaphragms, one or two drops of mercury should then be forced out of the tip of the shank to provide space for sealing in the pipette shaft.

In sealing the pipette shaft into the shank, the following method should be painstakingly observed: (*a*) see that the pipette shaft fits fairly closely the bore of the shank (Taylor, 1920); (*b*) draw out a 'stick' of softened de Khotinsky cement about half the diameter of a lead pencil and 2 or 3 inches long, one end of which should be tapered; (*c*) after carefully melting (*do not boil*) the tapered end of the 'stick,' draw a small ring of the melted cement around the pipette shank about 3 or 4 mm. from the end which is to be sealed into the shank, *and allow the ring to harden;* (*d*) heat the tip of the shank *just enough* to melt the ring of cement around the shaft when the latter is introduced into the shank. (Here again much caution should be exercised to avoid overheating the shank and so producing bubbles in the cement. The gas thus formed, escaping into the shank, seriously reduces the surface tension of the mercury as evidenced later by the breaking of the column of mercury in the pipette tip.) If these several steps in the procedure are duly observed, the de Khotinsky cement, which is by far the best I have tried, will by capillarity flow freely in between the pipette shaft and shank and fill *completely* this space, thus avoiding any air bubbles near the end of the shaft and the contamination of the mercury by gas from overheated cement.

Fairly satisfactory results have also been obtained from filling the shank by gravity with mercury (or water) through a capillary tube inserted into the narrow opening of the shank.

The pipette shaft having been sealed in place, the mercury is then forced to the tip of the pipette by turning *both* thumbscrews against the rubber diaphragms. The importance of utilizing two diaphragms in the system is understood upon recalling that means must be provided not only for forcing from the shank sufficient mercury to fill a pipette shaft of convenient length and bore, but also for effecting very delicate changes of the meniscus in the exceedingly fine pipette tip (having a lumen even less than 1 micron in diameter). Both requirements are surprisingly well met by means of two diaphragms and their opposing thumbscrews. Pressure is thereby afforded for sufficient range of movement without exceeding the extensile limits of the rubber and thus seriously impairing the production of minute and prompt changes of movement in the pipette tip. The most delicate changes, moreover, can be adequately effected only by equalizing the pressure of the two screws against their respective diaphragms. A micropipette of this pattern, which has been in use for more than six months, is, *without having been once refilled*, still in perfect working condition.

This micropipette device was designed especially to be supported and controlled on the new micromanipulator described above. Provision is thereby made not only for operating directly the pipette in the moist chamber on the microscope but fully as advantageously for changing pipette shafts quickly and safely without disturbing the microscope or moist-chamber. The device may be used, however, with other micromanipulators by providing a suitable clamp for supporting the metal parts and inserting a flexible metal or glass capillary in between the shank and a thick-walled glass capillary 3 or 4 inches long. The system must then be filled under vacuum. After sealing the pipette shaft into the thick-walled capillary, the latter is clamped into position in the micromanipulator. Such an arrangement is similar to that used by Chambers (1922) in setting up his Luer syringe device.

Transmitted March 11, 1925.

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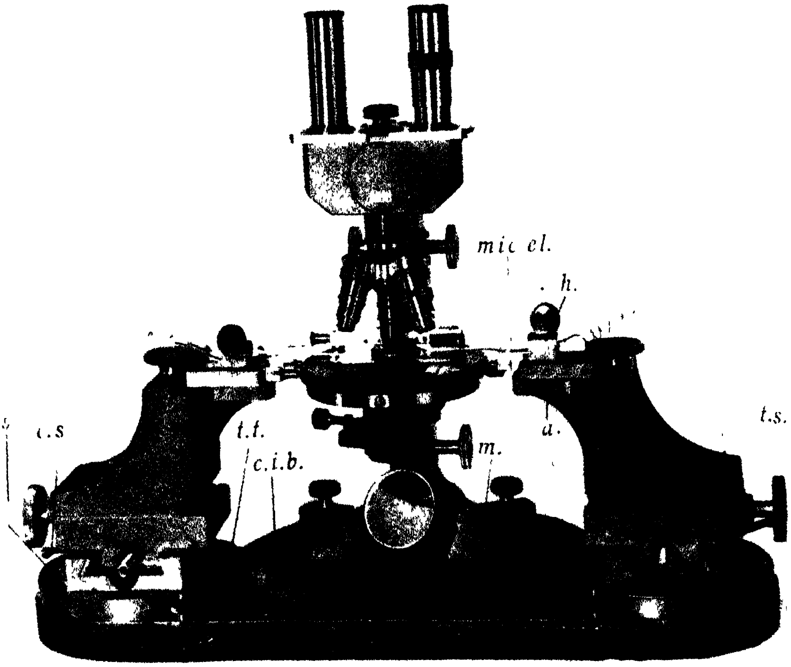
EXPLANATION OF PLATE

PLATE 34

The micromanipulator with microscope clamped in place: *a.*, arm supporting holder for operative instrument; *c.i.b.*, cast-iron base; *c.s.*, clampscrew for turntable; *h.*, holder for operative instrument; *m.*, clamp for microscope; *mic. el.*, microelectrode; *s.*, slot in turntable; *t.s.*, thumbscrew of manipulator; *t.t.*, turntable.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Page 31, description of fig. 15. *For* 8(16) *read* 6(12).

Page 145, line 7 from bottom. *For* four *read* three.

Page 182, line 5. *For* zone *read* zones.

Page 199, line 3. *For* Reticulotermes *read* Reticulitermes.

Page 199, line 12. *For* Reticulotermes hesperus *read* Reticulitermes flavipes.

Page 199, line 15. *For* Reticulotermes *read* Reticulitermes.

Page 212, line 6. *For* 52 *read* 25.

Page 227 [327], line 15. *For* probascis *read* proboscis.

In the paging of the papers by Raymund Millzner (Nos. 17 and 18) *read* 325-330 *for* 225–230.

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